

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/52, 9/00, 1/21 C12Q 1/68	A1	(11) International Publication Number: WO 94/08016 (43) International Publication Date: 14 April 1994 (14.04.94)
(21) International Application Number: PCT/US93/09340 (22) International Filing Date: 30 September 1993 (30.09.93) (30) Priority data: 07/956,700 2 October 1992 (02.10.92) US (71) Applicant: ARCH DEVELOPMENT CORPORATION [US/US]; 1101 E. 58th Street, Chicago, IL 60637 (US). (72) Inventors: HASELKORN, Robert ; 5834 S. Stony Island Avenue, Chicago, IL 60637 (US). GORNICKI, Piotr ; 1700 E. 56th Street, Apt. 1106, Chicago, IL 60637 (US). (74) Agents: NORTHRUP, Thomas, E. et al.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: CYANOBACTERIAL AND PLANT ACETYL-CoA CARBOXYLASE (57) Abstract The present invention provides isolated and purified polynucleotides that encode plant and cyanobacterial polypeptides that participate in the carboxylation of acetyl-CoA. Isolated cyanobacterial and plant polypeptides that catalyze acetyl-CoA carboxylation are also provided. Processes for altering acetyl-CoA carboxylation, increasing herbicide resistance of plants and identifying herbicide resistant variants of acetyl-CoA carboxylase are also provided.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CJ	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

CYANOBACTERIAL AND PLANT ACETYL-CoA CARBOXYLASE

Description

Technical Field of the Invention

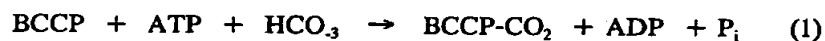
The present invention relates to polynucleotides and polypeptides of acetyl-CoA carboxylase in cyanobacteria and plants.

5 Polynucleotides encoding acetyl-CoA carboxylase have use in conferring herbicide resistance and in determining the herbicide resistance of plants in a breeding program.

Background of the Invention

10 Acetyl-CoA carboxylase (ACC) is the first enzyme of the biosynthetic pathway to fatty acids. It belongs to a group of carboxylases that use biotin as cofactor and bicarbonate as a source of the carboxyl group. ACC catalyzes the addition of CO₂ to acetyl-CoA to yield malonyl-CoA in two steps as shown below.

15



20

First, biotin becomes carboxylated at the expense of ATP. The carboxyl group is then transferred to Ac-CoA [Knowles, 1989]. This irreversible reaction is the committed step in fatty acid synthesis and is a target for multiple regulatory mechanisms. Reaction (1) is catalyzed by
25 biotin carboxylase (BC); reaction (2) by transcarboxylase (TC); BCCP = biotin carboxyl carrier protein.

ACC purified from *E. coli* contains three distinct, separable components.: biotin carboxylase (BC), a dimer of 49-kD monomers, biotin carboxyl carrier protein (BCCP) a dimer of 17-kD monomers and
30 transcarboxylase (TC), a tetramer containing two each of 33-kD and 35-kD subunits. The biotin prosthetic group is covalently attached to the γ -amino group of a lysine residue of BCCP. The primary structure of *E. coli* BCCP

-2-

and BC is known (*fabE* and *fabG* genes, respectively, have been cloned and sequenced) [Alix, 1989; Maramatsu, et al., 1989; Li, et al., 1992]. In bacteria, fatty acids are primarily precursors of phospholipids rather than storage fuels, and so ACC activity is coordinated with cell growth and division.

5 Rat and chicken ACC consist of a dimer of about 265 kD (rat has also a 280 kD isoform) subunits that contains all of the bacterial enzyme activities. Both mammalian and avian ACC are cytoplasmic enzymes and their substrate is transported out of mitochondria *via* citrate. ACC content and/or activity varies with the rate of fatty acid synthesis or energy requirements in different nutritional, hormonal and developmental states. ACC mRNA is transcribed using different promoters and can be regulated by alternative splicing. ACC catalytic activity is regulated allosterically by a number of metabolites and by reversible phosphorylation of the enzyme.

10 The primary structure of rat and chicken enzymes, and the primary structure of the 5'-untranslated region of mRNA have been deduced from cDNA sequences [Lopez-Casillas, et al., 1988; Takai, et al., 1988]. The primary structure of yeast ACC has also been determined [Feel, et al., 1992].

15 Studies on plant ACC are far less advanced [Harwood, 1988].

20 It was originally thought that plant ACC consisted of low molecular weight dissociable subunits similar to those of bacteria. Those results appeared to be due to degradation of the enzyme during purification. More recent results indicate that the wheat enzyme, as well as those from parsley and rape, are composed of two about 220 kD monomers, similar to the enzyme from rat and chicken [Harwood, 1988; Egin-Buhler, et al., 1983; Wurtelle, et al., 1990; Slabas, et al., 1985]. The plant ACC is located entirely in the stroma of plastids, where all plant fatty acid synthesis occurs. No plant gene encoding ACC has been reported to date. The gene must be nuclear because no corresponding sequence is seen in the complete chloroplast DNA

25 sequences of tobacco, liverwort or rice. ACC, like the vast majority of chloroplast proteins which are encoded in nuclear DNA, must be synthesized in the cytoplasm and then transported into the chloroplast, probably requiring

30

-3-

a chloroplast transport sequence. Although the basic features of plant ACC must be the same as those of prokaryotic and other eucaryotic ACCs, significant differences can be also expected due, for example, to differences in plant cell metabolism and ACC cellular localization.

5 Structural similarities deduced from the available amino acid sequences suggest strong evolutionary conservation among biotin carboxylases and biotin carboxylase domains of all biotin-dependent carboxylases. On the contrary, the BCCP domains show very little conservation outside the sequence E(A/V)MKM (lysine residue is
10 biotinylated) which is found in all biotinylated proteins including pyruvate carboxylase and propionyl-CoA carboxylase [Knowles, 1989; Samols, et al., 1988]. It is likely that the three functional domains of ACC located in *E.coli* on separate polypeptides are present in carboxylases containing two (human propionyl-CoA carboxylase) or only one (yeast pyruvate carboxylase,
15 mammalian, avian and probably also plant ACC) polypeptide as a result of gene fusion during evolution.

 Several years ago it was shown that aryloxyphenoxypropionates and cyclohexanediones, powerful herbicides effective against monocot weeds, inhibit fatty acid biosynthesis in sensitive
20 plants. Recently it has been determined that ACC is the target enzyme for both of these classes of herbicide. Dicotyledonous plants are resistant to these compounds, as are other eukaryotes and prokaryotes. The mechanisms of inhibition and resistance of the enzyme are not known [Lichtenthaler, 1990].

25 It has occurred to others that the evolutionary relatedness of cyanobacteria and plants make the former useful sources of cloned genes for the isolation of plant cDNAs. For example, Pecker et al used the cloned gene for the enzyme phytoene desaturase, which functions in the synthesis of carotenoids, from cyanobacteria as a probe to isolate the cDNA for that gene
30 from tomato [Pecker, et al., 1992].

Brief Summary of the Invention

-4-

In one aspect the present invention provides an isolated and purified polynucleotide of from about 1350 to about 40,000 base pairs that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium. Preferably, that
5 polypeptide is a subunit of acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA. In a preferred embodiment, a cyanobacterium is *Anabaena* or *Synechococcus*. The biotin carboxyl carrier protein preferably includes the amino acid residue sequence shown in SEQ ID NO:111 or a functional equivalent thereof.

10 In another preferred embodiment, the polypeptide has the amino acid residue sequence of Figure 1 or Figure 2. The polynucleotide preferably includes the DNA sequence of SEQ ID NO:1, the DNA sequence of SEQ ID NO:1 from about nucleotide position 1300 to about nucleotide position 2650 or the DNA sequence of SEQ ID NO:5.

15 In another aspect, the present invention provides an isolated and purified polynucleotide of from about 480 to about 40,000 base pairs that encodes a biotin carboxyl carrier protein of a cyanobacterium and, preferably *Anabaena*. The biotin carboxyl carrier protein preferably includes the amino acid residue sequence of SEQ ID NO:111 and the polynucleotide
20 preferably includes the DNA sequence of SEQ ID NO:110.

Another polynucleotide provided by the present invention encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA. A plant polypeptide is preferably (1) a monocotyledonous plant polypeptide such as a wheat, rice, maize, barley, rye, oats or timothy
25 grass polypeptide or (2) a dicotyledonous plant polypeptide such as a soybean, rape, sunflower, tobacco, *Arabidopsis*, petunia, Canola, pea, bean, tomato, potato, lettuce, spinach, alfalfa, cotton or carrot polypeptide. Preferably, that polypeptide is a subunit of ACC and participates in the
30 carboxylation of acetyl-CoA.

Such a polynucleotide preferably includes the nucleotide sequence of SEQ ID NO:108 and encodes the amino acid residue sequence of SEQ ID NO:109.

-5-

In yet another aspect, the present invention provides an isolated and purified DNA molecule comprising a promoter operatively linked to a coding region that encodes (1) a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium, (2) a biotin carboxyl carrier protein of a cyanobacterium or (3) a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby said promoter drives the transcription of said coding region.

In another aspect, the present invention provides an isolated polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium such as *Anabaena* or *Synechococcus*. Preferably a biotin carboxyl carrier protein includes the amino acid sequence of SEQ ID NO:111 and the polypeptide has the amino acid residue sequence of Figure 1 or Figure 2.

The present invention also provides (1) an isolated and purified biotin carboxyl carrier protein of a cyanobacterium such as *Anabaena*, which protein includes the amino acid residue sequence of SEQ ID NO:111 and (2) an isolated and purified plant polypeptide having a molecular weight of about 220 kD, dimers of which have the ability to catalyze the carboxylation of acetyl-CoA.

In yet another aspect, the present invention provides a process of increasing the herbicide resistance of a monocotyledonous plant comprising transforming the plant with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in a monocotyledonous plant.

Preferably, a polypeptide is an acetyl-CoA carboxylase enzyme and, more preferably, a dicotyledonous plant acetyl-CoA

-6-

carboxylase. In a preferred embodiment, a coding region includes the DNA sequence of SEQ ID NO:108 and a promoter is CaMV35.

5 The present invention also provides a transformed plant produced in accordance with the above process as well as a transgenic plant and a transgenic plant seed having incorporated into its genome a transgene that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA.

10 In yet another aspect, the present invention provides a process of altering the carboxylation of acetyl-CoA in a cell comprising transforming the cell with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in the cell.

15 In a preferred embodiment, a cell is a cyanobacterium or a plant cell and a plant polypeptide is a monocotyledonous plant acetyl-CoA carboxylase enzyme such as wheat acetyl-CoA carboxylase enzyme. The present invention also provides a transformed cyanobacterium produced in accordance with such a process.

20 The present invention still further provides a process for determining the inheritance of plant resistance to herbicides of the aryloxyphenocypionate or cyclohexanedione class, which process comprises the steps of:

- 25 (a) measuring resistance to herbicides of the aryloxyphenocypionate or cyclohexanedione class in a parental plant line and in progeny of the parental plant line;
- (b) purifying DNA from said parental plant line and the progeny;
- (c) digesting the DNA with restriction enzymes to form
- 30 DNA fragments;
- (d) fractionating the fragments on a gel;
- (e) transferring the fragments to a filter support;

-7-

(f) annealing the fragments with a labelled RFLP probe consisting of a DNA molecule that encodes acetyl-CoA carboxylase or a portion thereof; and

5 (g) detecting the presence of complexes between the fragments and the RFLP probe; and

(h) correlating the herbicide resistance of step (a) with the complexes of step (g) and thereby the inheritance of herbicide resistance.

Preferably, the acetyl-CoA carboxylase is a dicotyledonous plant acetyl-CoA carboxylase enzyme or a mutated monocotyledonous plant acetyl-CoA carboxylase that confers herbicide resistance or a hybrid acetyl-CoA carboxylase comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase, a portion of a dicotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

15 In still yet another aspect, the present invention provides a process for identifying herbicide resistant variants of a plant acetyl-CoA carboxylase comprising the steps of:

(a) transforming cyanobacteria with a DNA molecule that encodes a monocotyledonous plant acetyl-CoA carboxylase enzyme to form transformed cyanobacteria;

20 (b) inactivating cyanobacterial acetyl-CoA carboxylase;

(c) exposing the transformed cyanobacteria to a herbicide that inhibits acetyl-CoA carboxylase activity;

(d) identifying transformed cyanobacteria that are resistant to the herbicide; and

25 (e) characterizing DNA that encodes acetyl-CoA carboxylase from the cyanobacteria of step (d).

Brief Description of the Drawings

In the drawings which form a portion of the specification:

30 **Figure 1** shows the complete nucleotide sequence of a HindIII fragment that includes the fabG gene coding biotin carboxylase from the

-8-

cyanobacterium *Anabaena* 7120, along with the amino acid sequence deduced from the coding sequence of the DNA.

Figure 2 shows the nucleotide sequence of the coding region of the *fabG* gene from the cyanobacterium *Anacystis nidulans* R2, along with the amino acid sequence deduced from the coding sequence of the DNA.

Figure 3 shows an alignment of the amino acid sequences of the BC proteins from both cyanobacteria and from *E. coli*, the BCCP proteins from *Anabaena* and from *E. coli*, along with the ACC enzymes from rat and chicken and several other biotin-containing carboxylases. Stars indicate positions that are identical in all sequences or all but one. The conventional one letter abbreviations for amino acids are used. The BC domains are indicated by a solid underline, the BCCP domains by a dashed underline. The symbol # indicates sequences not related to BC and, therefore, not considered in the alignment. The wheat ACC sequence deduced from the sequence of our cloned cDNA fragment is on the top line. Abbreviations used in the Figure are: Wh ACC, wheat ACC; Rt, rat; Ch, chicken; Yt, yeast; Sy ACC, *Synechococcus* BC; An ACC, *Anabaena* BC and BCCP proteins; EC ACC, *E. coli* BC and BCCP; Hm PCCA, human propionyl CoA carboxylase; Rt PCCA, rat propionyl CoA carboxylase; Yt PC, yeast pyruvate carboxylase.

Figure 4 shows the conserved amino acid sequences used to design primers for the PCR to amplify the BC domain of ACC from wheat. The sequences of the oligonucleotide primers are also shown. In this and other figures showing primer sequences, A means adenine, C means cytosine, G means guanine, T means thymine, N means all four nucleotides, Y means T or C, R means A or G, K means G or T, M means A or C, W means A or T, and H means A, C or T.

Figure 5 shows the sequences of the oligonucleotides used as primers for the PCR used to amplify the region of wheat ACC cDNA between the BC and BCCP domains.

Figure 6 shows the nucleotide sequence of a portion of the wheat cDNA corresponding to ACC. The amino acid sequence deduced

-9-

from the nucleotide sequence is also shown. The underlined sequences correspond to the primer sites shown in Figure 5. A unique sequence was found for the BC domain, suggesting that a single mRNA was the template for the final amplified products. For the sequence between the BC and BCCP domains, three different variants were found among four products sequenced, suggesting that three different gene transcripts were among the amplified products. This is not unexpected because wheat is hexaploid, i.e. it has three pairs of each chromosome.

Figure 7 shows the sequences of the oligonucleotides used as primers to amplify most of the *fabE* gene encoding the biotin carboxyl carrier protein from DNA of *Anabaena*.

Figure 8 shows the nucleotide sequence of a PCR product corresponding to a portion of the *fabE* gene encoding about 75% of the biotin carboxyl carrier protein from the cyanobacterium *Anabaena*, along with the amino acid sequence deduced from the coding sequence. The underlined sequences correspond to the primer sites shown in Figure 7.

Detailed Description of the Invention

I. Definitions

The following words and phrases have the meanings set forth below.

Expression: The combination of intracellular processes, including transcription and translation undergone by a coding DNA molecule such as a structural gene to produce a polypeptide.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that provide an expression control element for a structural gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

Regeneration: The process of growing a plant from a plant cell (e.g. plant protoplast or explant).

Structural gene: A gene that is expressed to produce a polypeptide.

-10-

Transformation: A process of introducing an exogenous DNA sequence (e.g. a vector, a recombinant DNA molecule) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

5 **Transformed cell:** A cell whose DNA has been altered by the introduction of an exogenous DNA molecule into that cell.

Transgenic cell: Any cell derived or regenerated from a transformed cell or derived from a transgenic cell. Exemplary transgenic cells include plant calli derived from a transformed plant cell and particular
10 cells such as leaf, root, stem, e.g. somatic cells, or reproductive (germ) cells obtained from a transgenic plant.

Transgenic plant: A plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-
15 transgenic plant of the same strain. The terms "transgenic plant" and "transformed plant" have sometimes been used in the art as synonymous terms to define a plant whose DNA contains an exogenous DNA molecule. However, it is thought more scientifically correct to refer to a regenerated plant or callus obtained from a transformed plant cell or protoplast as being
20 a transgenic plant, and that usage will be followed herein.

Vector: A DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. A plasmid is an exemplary vector.

25 Certain polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a single letter or a three letter code as indicated below.

30

-11-

	<u>Amino Acid Residue</u>	<u>3-Letter Code</u>	<u>1-Letter Code</u>
	Alanine	Ala	A
	Arginine	Arg	R
5	Asparagine	Asn	N
	Aspartic Acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic Acid	Glu	E
10	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
15	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
20	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V

25 The present invention provides polynucleotides and
 polypeptides relating to a whole or a portion of acetyl-CoA carboxylase
 (ACC) of cyanobacteria and plants as well as processes using those
 polynucleotides and polypeptides.

30 II. Polynucleotides

As used herein the term "polynucleotide" means a sequence of
 nucleotides connected by phosphodiester linkages. A polynucleotide of the
 present invention can comprise from about 2 to about several hundred

-12-

thousand base pairs. Preferably, a polynucleotide comprises from about 5 to about 150,000 base pairs. Preferred lengths of particular polynucleotides are set hereinafter.

A polynucleotide of the present invention can be a deoxyribonucleic acid (DNA) molecule or a ribonucleic acid (RNA) molecule. Where a polynucleotide is a DNA molecule, that molecule can be a gene or a cDNA molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), and uracil (U).

A. Cyanobacteria

In one embodiment, the present invention contemplates an isolated and purified polynucleotide of from about 1350 to about 40,000 base pairs that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium.

Preferably, a biotin carboxyl carrier protein (BCCP) is derived from a cyanobacterium such as *Anabaena* or *Synechococcus*. A preferred *Anabaena* is *Anabaena* 7120. A preferred *Synechococcus* is *Anacystis nidulans* R2 (*Synechococcus* sp. strain pcc7942). A biotin carboxyl carrier protein preferably includes the amino acid residue sequence shown in SEQ ID NO:111 or a functional equivalent thereof.

Preferably, a polypeptide is a biotin carboxylase enzyme of a cyanobacterium, which enzyme is a subunit of acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA. In a preferred embodiment, a polypeptide encoded by such a polynucleotide has the amino acid residue sequence of Figure 1 or Figure 2, or a functional equivalent of those sequences.

A polynucleotide preferably includes the DNA sequence of SEQ ID NO:1 (Figure 1) or the DNA sequence of SEQ ID NO:1 (Figure 1) from about nucleotide position 1300 to about nucleotide position 2650.

The polynucleotide of SEQ ID NO:1 contains a gene that encodes the enzyme biotin carboxylase (BC) enzyme from the

-13-

5 cyanobacterium *Anabaena*. This gene was cloned in the following way: total DNA from *Anabaena* was digested with various restriction enzymes, fractionated by gel electrophoresis, and blotted onto GeneScreen Plus (DuPont). The blot was hybridized at low stringency (1 M NaCl, 57° C.) with a probe consisting of a SstII-PstI fragment containing about 90% of the coding region of the *fabG* gene from *E. coli*. This probe identified a 3.1-kb HindIII fragment in the *Anabaena* digest that contained similar sequences. A mixture of about 3-kb HindIII fragments of *Anabaena* DNA was purified, then digested with NheI, yielding a HindIII-NheI fragment of 1.6 kb that
10 hybridized with the *fabG* probe. The 1.6-kb region was purified by gel electrophoresis and cloned into pUC18.

Plasmid minipreps were made from about 160 colonies, of which four were found to contain the 1.6-kb HindIII-NheI fragment that hybridized with the *fabG* probe. The 1.6-kb *Anabaena* fragment was then
15 used as probe to screen, at high stringency (1 M NaCl, 65° C.), a cosmid library of *Anabaena* DNA inserts averaging 40 kb in size. Five were found among 1920 tested, all of which contained the same size HindIII and NheI fragments as those identified by the *E. coli* probe previously. From one of the cosmids, the 3.1-kb HindIII fragment containing the *Anabaena fabG* gene
20 was subcloned into pUC18 and sequenced using the dideoxy chain termination method. The complete nucleotide sequence of this fragment is shown in Figure 1.

A similar procedure was used to clone the *fabG* gene from *Synechococcus*. In this case, the initial Southern hybridization showed that
25 the desired sequences were contained in part on an 0.8-kb BamHI-PstI fragment. This size fragment was purified in two steps and cloned into the plasmid Bluescript KS. Minipreps of plasmids from 200 colonies revealed two that contained the appropriate fragment of *Synechococcus* DNA. This fragment was used to probe, at high stringency, a library of *Synechococcus*
30 inserts in the cosmid vector pWB79. One positive clone was found among 1728 tested. This cosmid contained a 2-kb BamHI and a 3-kb PstI fragment that had previously been identified by the *E. coli fabG* probe in digests of

-14-

total *Synechococcus* DNA. Both fragments were subcloned from the cosmid into Bluescript KS and 2.4 kb, including the coding part of the *fabG* gene, were sequenced. The complete sequence of the coding region of the *Synechococcus fabG* gene is shown in Figure 2.

5 In another aspect, the present invention provides an isolated and purified polynucleotide of from about 480 to about 40,000 base pairs that encodes a biotin carboxyl carrier protein of a cyanobacterium. That biotin carboxyl carrier protein preferably includes the amino acid residue sequence of Figure 8 (SEQ ID NO:111) or a functional equivalent thereof.
10 A preferred polynucleotide that encodes that polypeptide includes the DNA sequence of SEQ ID NO:110 (Figure 8).

B. Plants

Another polynucleotide contemplated by the present invention encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA. Such a plant polypeptide is preferably a monocotyledonous
15 or a dicotyledonous plant acetyl-CoA carboxylase enzyme.

An exemplary and preferred monocotyledonous plant is wheat, rice, maize, barley, rye, oats or timothy grass. An exemplary and preferred dicotyledonous plant is soybean, rape, sunflower, tobacco, *Arabidopsis*,
20 petunia, pea, Canola, bean, tomato, potato, lettuce, spinach, alfalfa, cotton or carrot.

A monocotyledonous plant polypeptide is preferably wheat ACC, which ACC includes the amino acid residue sequence of SEQ ID NO:109 (Figure 6) or a functional equivalent thereof. A preferred
25 polynucleotide that encodes such a polypeptide includes the DNA sequence of SEQ ID NO:108 (Figure 6).

Amino acid sequences of biotin carboxylase (BC) from *Anabaena* and *Synechococcus* show great similarity with amino acid residue sequences from other ACC enzymes as well as with the amino acid residue
30 sequences of other biotin-containing enzymes (See Figure 3). Based on that homology, the nucleotide sequences shown in Figure 4 were chosen for the construction of primers for polymerase chain reaction amplification of a

-15-

corresponding region of the gene for ACC from wheat. Those primers have the nucleotide sequences shown below:

Primer 1

5' TCGAATTCGTNATNATHAARGC 3' (SEQ ID NO:112);

5

Primer 2

5' GCTCTAGAGKRTGYTCNACYTG 3' (SEQ ID NO:113);

where N is A, C, G or T; H is A, C or T; R is A or G; Y is T or C and K is G or T. Primers 1 and 2 comprise a 14-nucleotide specific sequence based on a conserved amino acid sequence and an 8-nucleotide extension at the 5'-end of the primer to provide anchors for rounds of amplification after the first round and to provide convenient restriction sites for analysis and cloning.

10

cDNA amplification began with a preparation of total polyA-containing mRNA from eight day-old green plants (*Triticum aestivum* var. Era as described in [Lamppa, et al., 1992]). The first strand of cDNA was synthesized using random hexamers as primers for AMV reverse transcriptase following procedures described in [Haymerle, et al., 1986], with some modifications. Reverse transcriptase was inactivated by heat and low molecular weight material was removed by filtration.

15

20

The PCR was initiated by the addition of polymerase at 95°C. Amplification was for 45 cycles, each 1 min at 95°, 1 min at 42-46° and 2 min at 72° C. Both the reactions using *Anabaena* DNA and the single-stranded wheat cDNA as template yielded about 440 base pair (bp) products. The wheat product was eluted from a gel and reamplified using the same primers. That product, also 440 bp, was cloned into the Invitrogen (San Diego, CA) vector pCR1000 using their A/T tail method, and sequenced.

25

In eukaryotic ACCs, a BCCP domain is located about 300 amino acids away from the end of the BC domain, on the C-terminal side. Therefore, it is possible to amplify the cDNA covering the interval between the BC and BCCP domains using primers from the C-terminal end of the BC domain and the conserved MKM region of the BCCP. The BC primer was based on the wheat cDNA sequence obtained as described above. Those

30

-16-

primers, each with 6- or 8-base 5'-extensions, are shown below and in Figure 5.

Primer 3

5' GCTCTAGAATACTATTTTCCTG 3' (SEQ ID NO:114)

5

Primer 4

5' TCGAATTCWNCATYTTTCATNRC 3' (SEQ ID NO:115)

10

N, R and Y are as defined above. W is A or T. The BC primer (Primer 3) was based on the wheat cDNA sequence obtained as described above. The MKM primer (primer 4) was first checked by determining whether it would amplify the *fabE* gene coding BCCP from *Anabaena* DNA. This PCR was primed at the other end by using a primer based on the N-terminal amino acid residue sequence as determined on protein purified from *Anabaena* extracts by affinity chromatography. Those primers are shown below and in Figure 7.

15

Primer 5

5' GCTCTAGAYTTYAAAYGARATHMG 3' (SEQ ID NO:116)

Primer 4

5' TCGAATTCWNCATYTTTCATNRC 3' (SEQ ID NO:115)

20

H, N, R, T, Y and W are as defined above. M is A or C. This amplification (using the conditions described above) yielded the correct fragment of the *Anabaena fabE* gene, which was used to identify cosmids that contained the entire *fabE* gene and flanking DNA. An about 4 kb *Xba*I fragment containing the gene was cloned into the vector Bluescript KS for sequencing.

25

Primers 3 and 4 were then used to amplify the intervening sequence in wheat cDNA. Again, the product of the first PCR was eluted and reamplified by another round of PCR, then cloned into the Invitrogen vector pCRII.

30

The complete 1.1 kb of the amplified DNA was sequenced, shown in Figure 6, nucleotides 376-1473. The nucleotide sequence of the BC domain is also shown in Figure 6, nucleotides 1-422. Three clones of

-17-

the BC domain gave the sequence shown. Four clones of the 1.1-kb fragment differed at several positions, corresponding to three closely related sequences, all of which are indicated in the Figure. Most of the sequence differences are in the third codon position and are silent in terms of the amino acid sequence.

The amino acid sequence of the polypeptide predicted from the cDNA sequence for this entire fragment of wheat cDNA (1473 nucleotides) is compared with the amino acid sequences of other ACC enzymes and related enzymes from various sources in Figure 3. The most significant identities are with the ACC of rat, chicken and yeast, as shown in the table below. Less extensive similarities are evident with the BC subunits of bacteria and the BC domains of other enzymes such as pyruvate carboxylase of yeast and propionyl CoA carboxylase of rat. The amino acid identities between wheat ACC and other biotin-dependent enzymes, within the BC domain (amino acid residues 312-630 in Figure 3) are shown below in Table 1.

-18-

Table 1

	<u>% identity with wheat ACC</u>	<u>% identity with rat ACC</u>
5		
	rat ACC	58 (100)
	chicken ACC	57
	yeast ACC	56
	<i>Synechococcus</i> ACC	32
10	<i>Anabaena</i> ACC	30
	<i>E. coli</i> ACC	33
	rat propionyl CoA carboxylase	32 31
15	yeast pyruvate carboxylase	31

C. Probes and Primers

In another aspect, DNA sequence information provided by the invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences of the selected polynucleotides disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared based on a consideration of a selected ACC gene sequence, e.g., a sequence such as that shown in Figures 1, 2, 6 or 8. The ability of such nucleic acid probes to specifically hybridize to an ACC gene sequence lend them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of an ACC gene from a cyanobacterium or a plant using PCR technology. Segments of ACC genes from other organisms can also be amplified by PCR using such primers.

-19-

To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes sequences that are complementary to at least a 10 to 30 or so long nucleotide stretch of an ACC sequence, such as that shown in Figures 1, 2, 6 or 8. A size of at least 10 nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology of U.S. Patent 4,603,102, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction sites.

Accordingly, a nucleotide sequence of the invention can be used for its ability to selectively form duplex molecules with complementary stretches of the gene. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degree of selectivity of the probe toward the target sequence. For applications requiring a high degree of selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, for example, one will select relatively low salt and/or high temperature conditions, such as provided by 0.02M-0.15M NaCl at temperatures of 50°C to 70°C. These conditions are particularly selective, and tolerate little, if any, mismatch between the probe and the template or target strand.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate an ACC coding

-20-

sequences for related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as 0.15M-0.9M salt, at temperatures ranging from 20°C to 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it is advantageous to employ a polynucleotide of the present invention in combination with an appropriate label for detecting hybrid formation. A wide variety of appropriate labels are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal.

In general, it is envisioned that a hybridization probe described herein is useful both as a reagent in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions depend as is well known in the art on the particular circumstances and criteria required (e.g., on the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe). Following washing of the matrix to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label.

D. Expression Vector

The present invention contemplates an expression vector comprising a polynucleotide of the present invention. Thus, in one embodiment an expression vector is an isolated and purified DNA molecule

-21-

comprising a promoter operatively linked to an coding region that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium, which coding region is operatively linked to a transcription-terminating region, whereby the promoter drives the transcription of the coding region.

As used herein, the term "operatively linked" means that a promoter is connected to an coding region in such a way that the transcription of that coding region is controlled and regulated by that promoter. Means for operatively linking a promoter to a coding region are well known in the art.

Where an expression vector of the present invention is to be used to transform a cyanobacterium, a promoter is selected that has the ability to drive and regulate expression in cyanobacteria. Promoters that function in bacteria are well known in the art. An exemplary and preferred promoter for the cyanobacterium *Anabaena* is the *glnA* gene promoter. An exemplary and preferred promoter for the cyanobacterium *Synechococcus* is the *psbAI* gene promoter. Alternatively, the cyanobacterial *fabG* gene promoters themselves can be used.

Where an expression vector of the present invention is to be used to transform a plant, a promoter is selected that has the ability to drive expression in plants. Promoters that function in plants are also well known in the art. Useful in expressing the polypeptide in plants are promoters that are inducible, viral, synthetic, constitutive as described by Poszkowski et al., EMBO J., 3:2719 (1989) and Odell et al., Nature, 313:810 (1985), and temporally regulated, spatially regulated, and spatiotemporally regulated as given in Chau et al., Science, 244:174-181 (1989).

A promoter is also selected for its ability to direct the transformed plant cell's or transgenic plant's transcriptional activity to the coding region. Structural genes can be driven by a variety of promoters in plant tissues. Promoters can be near-constitutive, such as the CaMV 35S promoter, or tissue specific or developmentally specific promoters affecting dicots or monocots.

-22-

Where the promoter is a near-constitutive promoter such as CaMV 35S, increases in polypeptide expression are found in a variety of transformed plant tissues (e.g. callus, leaf, seed and root). Alternatively, the effects of transformation can be directed to specific plant tissues by using plant integrating vectors containing a tissue-specific promoter.

An exemplary tissue-specific promoter is the Lectin promoter, which is specific for seed tissue. The Lectin protein in soybean seeds is encoded by a single gene (Le1) that is only expressed during seed maturation and accounts for about 2 to about 5 percent of total seed mRNA. The Lectin gene and seed-specific promoter have been fully characterized and used to direct seed specific expression in transgenic tobacco plants. See, e.g., Vodkin et al., Cell, 34:1023 (1983) and Lindstrom et al., Developmental Genetics, 11:160 (1990).

An expression vector containing a coding region that encodes a polypeptide of interest is engineered to be under control of the Lectin promoter and that vector is introduced into plants using, for example, a protoplast transformation method. Dhir et al., Plant Cell Reports, 10:97 (1991). The expression of the polypeptide is directed specifically to the seeds of the transgenic plant.

A transgenic plant of the present invention produced from a plant cell transformed with a tissue specific promoter can be crossed with a second transgenic plant developed from a plant cell transformed with a different tissue specific promoter to produce a hybrid transgenic plant that shows the effects of transformation in more than one specific tissue.

Exemplary tissue-specific promoters are corn sucrose synthetase 1 (Yang et al. Proc. Natl. Acad. Sci. U.S.A., 87:4144-48 (1990)), corn alcohol dehydrogenase 1 (Vogel et al., J. Cell Biochem., (supplement 13D, 312) (1989)), corn zein 19KD gene (storage protein) (Boston et al., Plant Physiol., 83:742-46), corn light harvesting complex (Simpson, Science, 233:34 (1986), corn heat shock protein (O'Dell et al., Nature, 313:810-12 (1985), pea small subunit RuBP Carboxylase (Poulsen et

-23-

al., Mol. Gen. Genet., 205:193-200 (1986); Cashmore et al., Gen. Eng. of Plants, Plenum Press, New York, 29-38 (1983), Ti plasmid mannopine synthase (Langridge et al., Proc. Natl. Acad. Sci. USA, 86:3219-3223 (1989), Ti plasmid nopaline synthase (Langridge et al., Proc. Natl. Acad. Sci. USA, 86:3219-3223 (1989), petunia chalcone isomerase (Van Tunen et al., EMBO J., 7:1257 (1988), bean glycine rich protein 1 (Keller et al., EMBO J., 8:1309-14 (1989), CaMV 35s transcript (O'Dell et al., Nature, 313:810-12 (1985) and Potato patatin (Wenzler et al., Plant Mol. Biol., 12:41-50 (1989). Preferred promoters are the cauliflower mosaic virus (CaMV 35S) promoter and the S-E9 small subunit RuBP carboxylase promoter.

The choice of which expression vector and ultimately to which promoter a polypeptide coding region is operatively linked depends directly on the functional properties desired, e.g. the location and timing of protein expression, and the host cell to be transformed. These are well known limitations inherent in the art of constructing recombinant DNA molecules. However, a vector useful in practicing the present invention is capable of directing the expression of the polypeptide coding region to which it is operatively linked.

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers et al., Meth. in Enzymol., 153:253-277 (1987). However, several other plant integrating vector systems are known to function in plants including pCaMVCN transfer control vector described by Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985). Plasmid pCaMVCN (available from Pharmacia, Piscataway, NJ) includes the cauliflower mosaic virus CaMV 35S promoter.

In preferred embodiments, the vector used to express the polypeptide includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin resistance; i.e.,

the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II and nopaline synthase 3' nontranslated region described by Rogers et al., in Methods For Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press Inc., San Diego, CA
5 (1988).

RNA polymerase transcribes a coding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as
10 transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA).

Means for preparing expression vectors are well known in the art. Expression (transformation vectors) used to transform plants and methods of making those vectors are described in United States Patent Nos.
15 4,971,908, 4,940,835, 4,769,061 and 4,757,011, the disclosures of which are incorporated herein by reference. Those vectors can be modified to include a coding sequence in accordance with the present invention.

A variety of methods has been developed to operatively link DNA to vectors via complementary cohesive termini or blunt ends. For
20 instance, complementary homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

A coding region that encodes a polypeptide having the ability
25 to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium is preferably a biotin carboxylase enzyme of a cyanobacterium, which enzyme is a subunit of acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA. In a preferred embodiment, such a polypeptide has the amino acid residue sequence of Figure 1 or
30 Figure 2, or a functional equivalent of those sequences. In accordance with such an embodiment, a coding region comprises the entire DNA sequence of SEQ ID NO:1 (Figure 1) or the DNA sequence of SEQ ID NO:1 (Figure 1)

-25-

from about nucleotide position 1300 to about nucleotide position 2650 or the DNA sequence of SEQ ID NO:5 (Figure 2).

5 In another embodiment, an expression vector comprises a coding region of from about 480 to about 40,000 base pairs that encodes a biotin carboxyl carrier protein of a cyanobacterium. That biotin carboxyl carrier protein preferably includes the amino acid residue sequence of Figure 8 (SEQ ID NO:111) or a functional equivalent thereof. A preferred such coding region includes the DNA sequence of SEQ ID NO:110 (Figure 8).

10 In still yet another embodiment, an expression vector comprises a coding region that encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA. Such a plant polypeptide is preferably a monocotyledonous or a dicotyledonous plant acetyl-CoA carboxylase enzyme.

15 A preferred monocotyledonous plant polypeptide encoded by such a coding region is preferably wheat ACC, which ACC includes the amino acid residue sequence of SEQ ID NO:109 (Figure 6) or a functional equivalent thereof. A preferred coding region includes the DNA sequence of SEQ ID NO:108 (Figure 6).

20 III. Polypeptide

The present invention contemplates a polypeptide that defines a whole or a portion of an ACC of a cyanobacterium or a plant. In one embodiment, thus, the present invention provides an isolated polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium such as *Anabaena* or *Synechococcus*.
25 Preferably, a biotin carboxyl carrier protein includes the amino acid sequence of SEQ ID NO:111 and the polypeptide has Figure 1 or Figure 2.

The present invention also contemplates an isolated and purified biotin carboxyl carrier protein of a cyanobacterium such as
30 *Anabaena*, which protein includes the amino acid residue sequence of SEQ ID NO:111.

-26-

In another embodiment, the present invention contemplates an isolated and purified plant polypeptide having a molecular weight of about 220 KD, dimers of which have the ability to catalyze the carboxylation of acetyl-CoA. Such a polypeptide preferably includes the amino acid residue sequence of SEQ ID NO:109.

5
10
15
Modification and changes may be made in the structure of polypeptides of the present invention and still obtain a molecule having like or otherwise desirable characteristics. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like or even countervailing properties (e.g., antagonistic v. agonistic).

20
25
30
In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte & Doolittle, J. Mol. Biol., 157:105-132, 1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide,

-27-

which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid may be substituted by another amino acid having a similar hydropathic index and still obtain a biological functionally equivalent protein. In such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biological functional equivalent protein or peptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size,

-28-

and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

5 The present invention thus contemplates functional equivalents of the polypeptides set forth above. A polypeptide of the present invention is prepared by standard techniques well known to those skilled in the art. Such techniques include, but are not limited to, isolation and purification from tissues known to contain that polypeptide and expression from cloned
10 DNA using transformed cells.

IV. Transformed or transgenic cells or plants

A cyanobacterium, a plant cell or a plant transformed with an expression vector of the present invention is also contemplated. A
15 transgenic cyanobacterium, plant cell or plant derived from such a transformed or transgenic cell is also contemplated.

Means for transforming cyanobacteria are well known in the art. Typically, means of transformation are similar to those well known means used to transform other bacteria such as *E. coli*. *Synechococcus* can
20 be transformed simply by incubation of log-phase cells with DNA. (Golden, et al., 1987)

The application of brief, high-voltage electric pulses to a variety of mammalian and plant cells leads to the formation of nanometer-sized pores in the plasma membrane. DNA is taken directly into the cell
25 cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation can be extremely efficient and can be used both for transient expression of clones genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast
30 to calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

-29-

Methods for DNA transformation of plant cells include *Agrobacterium*-mediated plant transformation, protoplast transformation, gene transfer into pollen, injection into reproductive organs, injection into immature embryos and particle bombardment. Each of these methods has distinct advantages and disadvantages. Thus, one particular method of introducing genes into a particular plant strain may not necessarily be the most effective for another plant strain, but it is well known which methods are useful for a particular plant strain.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described by Fraley et al., Biotechnology, 3:629 (1985) and Rogers et al., Methods in Enzymology, 153:253-277 (1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome as described by Spielmann et al., Mol. Gen. Genet., 205:34 (1986) and Jorgensen et al., Mol. Gen. Genet., 207:471 (1987).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described by Klee et al., in Plant DNA Infectious Agents, T. Hohn and J. Schell, eds., Springer-Verlag, New York (1985) pp. 179-203.

Moreover, recent technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described by Rogers et al., Methods in Enzymology, 153:253 (1987), have convenient multi-linker regions flanked by a promoter and a polyadenylation

-30-

site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, *Agrobacteria* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

Agrobacterium-mediated transformation of leaf disks and other tissues such as cotyledons and hypocotyls appears to be limited to plants that *Agrobacterium* naturally infects. *Agrobacterium*-mediated transformation is most efficient in dicotyledonous plants. Few monocots appear to be natural hosts for *Agrobacterium*, although transgenic plants have been produced in asparagus using *Agrobacterium* vectors as described by Bytebier et al., Proc. Natl. Acad. Sci. USA, 84:5345 (1987). Therefore, commercially important cereal grains such as rice, corn, and wheat must usually be transformed using alternative methods. However, as mentioned above, the transformation of asparagus using *Agrobacterium* can also be achieved. See, for example, Bytebier, et al., Proc. Natl. Acad. Sci. USA, 84:5345 (1987).

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. However, inasmuch as use of the word "heterozygous" usually implies the presence of a complementary gene at the same locus of the second chromosome of a pair of chromosomes, and there is no such gene in a plant containing one added gene as here, it is believed that a more accurate name for such a plant is an independent segregant, because the added, exogenous gene segregates independently during mitosis and meiosis.

More preferred is a transgenic plant that is homozygous for the added structural gene; i.e., a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the

-31-

resulting plants produced for enhanced carboxylase activity relative to a control (native, non-transgenic) or an independent segregant transgenic plant.

It is to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments. See, for example, Potrykus et al., Mol. Gen. Genet., 199:183 (1985); Lorz et al., Mol. Gen. Genet., 199:178 (1985); Fromm et al., Nature, 319:791 (1986); Uchimiya et al., Mol. Gen. Genet., 204:204 (1986); Callis et al., Genes and Development, 1:1183 (1987); and Marcotte et al., Nature, 335:454 (1988).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described in Fujimura et al., Plant Tissue Culture Letters, 2:74 (1985); Toriyama et al., Theor Appl. Genet., 73:16 (1986); Yamada et al., Plant Cell Rep., 4:85 (1986); Abdullah et al., Biotechnology, 4:1087 (1986).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described by Vasil, Biotechnology, 6:397 (1988). In addition, "particle gun" or high-velocity microprojectile technology can be utilized. (Vasil, 1992)

Using that latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described in Klein et al., Nature, 327:70 (1987); Klein et al., Proc. Natl. Acad. Sci. U.S.A., 85:8502 (1988); and McCabe et al., Biotechnology,

-32-

6:923 (1988). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Metal particles have been used to successfully transform corn cells and to produce fertile, stable transgenic tobacco plants as described by
5 Gordon-Kamm, W.J. et al., The Plant Cell, 2:603-618 (1990); Klein, T.M. et al., Plant Physiol., 91:440-444 (1989); Klein, T.M. et al., Proc. Natl. Acad. Sci. USA, 85:8502-8505 (1988); and Tomes, D.T. et al., Plant Mol. Biol., 14:261-268 (1990). Transformation of tissue explants eliminates the need for passage through a protoplast stage and thus speeds the production of
10 transgenic plants.

Thus, the amount of a gene coding for a polypeptide of interest (i.e., a polypeptide having carboxylation activity) can be increased in monocotyledonous plants such as corn by transforming those plants using particle bombardment methods. Maddock et al., Third International
15 Congress of Plant Molecular Biology, Abstract 372 (1991). By way of example, an expression vector containing an coding region for a dicotyledonous ACC and an appropriate selectable marker is transformed into a suspension of embryonic maize (corn) cells using a particle gun to deliver the DNA coated on microprojectiles. Transgenic plants are
20 regenerated from transformed embryonic calli that express ACC. Particle bombardment has been used to successfully transform wheat (Vasil et al., 1992).

DNA can also be introduced into plants by direct DNA transfer into pollen as described by Zhou et al., Methods in Enzymology,
25 101:433 (1983); D. Hess, Intern Rev. Cytol., 107:367 (1987); Luo et al., Plant Mol. Biol. Reporter, 6:165 (1988). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described by Pena et al., Nature, 325:274 (1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of
30 desiccated embryos as described by Neuhaus et al., Theor. Appl. Genet., 75:30 (1987); and Benbrook et al., in Proceedings Bio Expo 1986, Butterworth, Stoneham, MA, pp. 27-54 (1986).

-33-

The development or regeneration of plants from either single plant protoplasts or various explants is well known in the art. See, for example, Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, CA (1988). This
5 regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth
10 medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described by Horsch et al., Science, 227:1229-1231 (1985).
15 In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described by Fraley et al., Proc. Natl. Acad. Sci. U.S.A., 80:4803 (1983).

This procedure typically produces shoots within two to four
20 months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain
25 employed, such variations being well known in the art.

Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants, as discussed before. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen
30 from plants of those important lines is used to pollinate regenerated plants.

A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in

-34-

the art. Any of the transgenic plants of the present invention can be cultivated to isolate the desired ACC or fatty acids which are the products of the series of reactions of which that catalyzed by ACC is the first.

5 A transgenic plant of this invention thus has an increased amount of an coding region (e.g. gene) that encodes a polypeptide of interest. A preferred transgenic plant is an independent segregant and can transmit that gene and its activity to its progeny. A more preferred transgenic plant is homozygous for that gene, and transmits that gene to all of its offspring on sexual mating.

10 Seed from a transgenic plant is grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for, by way of example, herbicide resistance, preferably in the field, under a range of environmental conditions.

15 The commercial value of a transgenic plant with increased herbicide resistance or with altered fatty acid production is enhanced if many different hybrid combinations are available for sale. The user typically grows more than one kind of hybrid based on such differences as time to maturity, standability or other agronomic traits. Additionally, hybrids adapted to one part of a country are not necessarily adapted to another part because of differences in such traits as maturity, disease and herbicide resistance. Because of this, herbicide resistance is preferably bred into a large number of parental lines so that many hybrid combinations can be produced.

V. Process of increasing herbicide resistance

Herbicides such as aryloxyphenoxypropionates and cyclohexanediones inhibit the growth of monocotyledonous weeds by
30 interfering with fatty acid biosynthesis of herbicide sensitive plants. ACC is the target enzyme for those herbicides. Dicotyledonous plants, other

-35-

eukaryotic organisms and prokaryotic organisms are resistant to those compounds.

Thus, the resistance of sensitive monocotyledonous plants to herbicides can be increased by providing those plants with ACC that is not sensitive to herbicide inhibition. The present invention therefore provides a process of increasing the herbicide resistance of a monocotyledonous plant comprising transforming the plant with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in a monocotyledonous plant.

Preferably, a herbicide resistant polypeptide, a dicotyledonous plant polypeptide such as an acetyl-CoA carboxylase enzyme from soybean, rape, sunflower, tobacco, *Arabidopsis*, petunia, Canola, pea, bean, tomato, potato, lettuce, spinach, alfalfa, cotton or carrot, or functional equivalent thereof. A promoter and a transcription-terminating region are preferably the same as set forth above.

Transformed monocotyledonous plants can be identified using herbicide resistance. A process for identifying a transformed monocotyledonous plant cell comprises the steps of:

- (a) transforming the monocotyledonous plant cell with a DNA molecule that encodes a dicotyledonous acetyl-CoA carboxylase enzyme; and
- (b) determining the resistance of the plant cell to a herbicide and thereby the identification of the transformed monocotyledonous plant cell.

Means for transforming a monocotyledonous plant cell are the same as set forth above.

The resistance of a transformed plant cell to a herbicide is preferably determined by exposing such a cell to an effective herbicidal dose of a preselected herbicide and maintaining that cell for a period of time and

-36-

under culture conditions sufficient for the herbicide to inhibit ACC, alter fatty acid biosynthesis or retard growth. The effects of the herbicide can be studied by measuring plant cell ACC activity, fatty acid synthesis or growth.

An effective herbicidal dose of a given herbicide is that amount of the herbicide that retards growth or kills plant cells not containing herbicide-resistant ACC or that amount of a herbicide known to inhibit plant growth. Means for determining an effective herbicidal dose of a given herbicide are well known in the art. Preferably, a herbicide used in such a process is an aryloxyphenoxypropionate or cyclohexanedione herbicide.

10

VI. Process of altering ACC activity

Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA. Thus, the carboxylation of acetyl-CoA in a cyanobacterium or a plant can be altered by, for example, increasing an ACC gene copy number or changing the composition (e.g., nucleotide sequence) of an ACC gene. Changes in ACC gene composition can alter gene expression at either the transcriptional or translational level. Alternatively, changes in gene composition can alter ACC function (e.g., activity, binding) by changing primary, secondary or tertiary structure of the enzyme. By way of example, certain changes in ACC structure are associated with changes in the resistance of that altered ACC to herbicides. The copy number of such a gene can be increased by transforming a cyanobacterium or a plant cell with an appropriate expression vector comprising a DNA molecule that encodes ACC.

15

20

25

30

In one embodiment, therefore, the present invention contemplates a process of altering the carboxylation of acetyl-CoA in a cell comprising transforming the cell with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in the cyanobacterium.

-37-

In a preferred embodiment, a cell is a cyanobacterium or a plant cell, a polypeptide is a cyanobacterial ACC or a plant ACC. Exemplary and preferred expression vectors for use in such a process are the same as set forth above.

5 Where a cyanobacterium is transformed with a plant ACC DNA molecule, that cyanobacterium can be used to identify herbicide resistant mutations in the gene encoding ACC. In accordance with such a use, the present invention provides a process for identifying herbicide resistant variants of a plant acetyl-CoA carboxylase comprising the steps of:

10 (a) transforming cyanobacteria with a DNA molecule that encodes a monocotyledonous plant acetyl-CoA carboxylase enzyme to form transformed or transfected cyanobacteria;

 (b) inactivating cyanobacterial acetyl-CoA carboxylase;

 (c) exposing the transformed cyanobacteria to an effective
15 herbicidal amount of a herbicide that inhibits acetyl-CoA carboxylase activity;

 (d) identifying transformed cyanobacteria that are resistant to the herbicide; and

 (e) characterizing DNA that encodes acetyl-CoA
20 carboxylase from the cyanobacteria of step (d).

 Means for transforming cyanobacteria as well as expression vectors used for such transformation are preferably the same as set forth above. In a preferred embodiment, cyanobacteria are transformed or transfected with an expression vector comprising an coding region that
25 encodes wheat ACC.

 Cyanobacteria resistant to the herbicide are identified. Identifying comprises growing or culturing transformed cells in the presence of the herbicide and recovering those cells that survive herbicide exposure.

 Transformed, herbicide-resistant cells are then grown in
30 culture, collected and total DNA extracted using standard techniques. ACC DNA is isolated, amplified if needed and then characterized by comparing that DNA with DNA from ACC known to be inhibited by that herbicide.

VII. Process for Determining Herbicide Resistance

Inheritability

In yet another aspect, the present invention provides a process for determining the inheritance of plant resistance to herbicides of the aryloxyphenocyclopropanate or cyclohexanedione class. That process comprises the steps of:

- (a) measuring resistance to herbicides of the aryloxyphenocyclopropanate or cyclohexanedione class in a parental plant line and in progeny of the parental plant line to;
- (b) purifying DNA from the parental plant line and the progeny;
- (c) digesting the DNA with restriction enzymes to form DNA fragments;
- (d) fractionating the fragments on a gel;
- (e) transferring the fragments to a filter support;
- (f) annealing the fragments with a labelled RFLP probe consisting of a DNA molecule that encodes acetyl-CoA carboxylase or a portion thereof;
- (g) detecting the presence of complexes between the fragments and the RFLP probe; and
- (h) correlating the herbicide resistance of step (a) with the complexes of step (g) and thereby the inheritance of herbicide resistance.

In a preferred embodiment, the herbicide resistant variant of acetyl-CoA carboxylase is a dicotyledonous plant acetyl-CoA carboxylase enzyme or a portion thereof. In another preferred embodiment, the herbicide resistant variant of acetyl-CoA carboxylase is a mutated monocotyledonous plant acetyl-CoA carboxylase that confers herbicide resistance or a hybrid acetyl-CoA carboxylase comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase, a portion of a dicotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

-39-

The inheritability of phenotypic traits such as herbicide resistance can be determined using RFLP analysis. Restriction fragment length polymorphisms (RFLPs) are due to sequence differences detectable by lengths of DNA fragments generated by digestion with restriction enzymes and typically revealed by agarose gel electrophoresis. There are large numbers of restriction endonucleases available, characterized by their recognition sequences and source.

Restriction fragment length polymorphism analyses are conducted, for example, by Native Plants Incorporated (NPI). This service is available to the public on a contractual basis. For this analysis, the genetic marker profile of the parental inbred lines is determined. If parental lines are essentially homozygous at all relevant loci (i.e., they should have only one allele at each locus), the diploid genetic marker profile of the hybrid offspring of the inbred parents should be the sum of those parents, e.g., if one parent had the allele A at a particular locus, and the other parent had B, the hybrid AB is by inference.

Probes capable of hybridizing to specific DNA segments under appropriate conditions are prepared using standard techniques well known to those skilled in the art. The probes are labelled with radioactive isotopes or fluorescent dyes for ease of detection. After restriction fragments are separated by size, they are identified by hybridization to the probe. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLP's are co-dominant alleles, thereby satisfying a criteria for a genetic marker. They differ from some other types of markers, e.g., from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation. Furthermore, different RFLP profiles result from different arrays of restriction endonucleases.

The foregoing examples illustrate particular embodiments of the present invention. It will be readily apparent to a skilled artisan that changes, modification and alterations can be made to those embodiments without departing from the true scope or spirit of the invention.

-40-

Example 1: Isolation of Cyanobacterial ACC Polynucleotides

The polynucleotide of SEQ ID NO:1 contains a gene that encodes the enzyme biotin carboxylase (BC) enzyme from the cyanobacterium *Anabaena* 7120. This gene was cloned from a total DNA
5 extract of *Anabaena* that was digested with various restriction enzymes, fractionated by gel electrophoresis, and blotted onto GeneScreen Plus (DuPont).

The blot was hybridized at low stringency (1 M NaCl, 57° C.) with a probe consisting of a SstII-PstI fragment containing about 90% of
10 the coding region of the *fabG* gene from *E. coli*. This probe identified a 3.1-kb HindIII fragment in the *Anabaena* digest that contained similar sequences. A mixture of about 3-kb HindIII fragments of *Anabaena* DNA was purified, then digested with NheI, yielding a HindIII-NheI fragment of 1.6 kb that hybridized with the *fabG* probe. The 1.6-kb region was purified
15 by gel electrophoresis and cloned into pUC18. Plasmid minipreps were made from about 160 colonies, of which four were found to contain the 1.6-kb HindIII-NheI fragment that hybridized with the *fabG* probe. The 1.6-kb *Anabaena* fragment was then used as probe to screen, at high stringency (1 M NaCl, 65° C.), a cosmid library of *Anabaena* DNA inserts averaging 40
20 kb in size. Five were found among 1920 tested, all of which contained the same size HindIII and NheI fragments as those identified by the *E. coli* probe previously. From one of the cosmids, the 3.1-kb HindIII fragment containing the *Anabaena fabG* gene was subcloned into pUC18 and sequenced using the dideoxy chain termination method. The complete
25 nucleotide sequence of this fragment is shown in Figure 1.

A similar procedure was used to clone the *fabG* gene from *Synechococcus*. In this case, the initial Southern hybridization showed that the desired sequences were contained in part on an 0.8-kb BamHI-PstI
30 fragment. This size fragment was purified in two steps and cloned into the plasmid Bluescript KS. Minipreps of plasmids from 200 colonies revealed two that contained the appropriate fragment of *Synechococcus* DNA. This fragment was used to probe, at high stringency, a library of *Synechococcus*

-41-

inserts in the cosmid vector pWB79. One positive clone was found among 1728 tested. This cosmid contained a 2-kb BamHI and a 3-kb PstI fragment that had previously been identified by the *E. coli fabG* probe in digests of total *Synechococcus* DNA. Both fragments were subcloned from the cosmid
5 into Bluescript KS and 2.4 kb, including the coding part of the *fabG* gene, were sequenced. The complete sequence of the coding region of the *Anacystis fabG* gene is shown in Figure 2.

Example 2: Plant ACC

10 The amino acid sequences of the *fabG* genes encoding BC from *Anabaena* and *Synechococcus* are aligned with sequences of ACC and other biotin-containing enzymes from several sources in Figure.3. This comparison allows the designation of several areas of significant conservation among all the proteins, indicated by stars in the Figure. Based
15 on this alignment, the sequences shown in Figure 4 were chosen for the construction of primers for the polymerase chain reaction, in order to amplify the corresponding region of the gene for ACC from wheat. The primers used for this amplification are shown in Figure 4. Each consists of a 14-nucleotide specific sequence based on the amino acid sequence and an
20 8-nucleotide extension at the 5'-end of the primer to provide anchors for rounds of amplification after the first round and to provide convenient restriction sites for future analysis and cloning.

cDNA amplification began with a preparation of total polyA-containing mRNA from eight day-old green plants (*Triticum aestivum* var. Era as described in [Lamppa, et al., 1992]). The first strand of cDNA was
25 synthesized using random hexamers as primers for AMV reverse transcriptase following procedures described in [Haymerle, et al., 1986], with some modifications. Reverse transcriptase was inactivated by incubation at 90° C and low molecular weight material was removed by
30 filtration through centricon 100. All components of the PCR (from the Cetus/Perkin-Elmer kit) together with the two primers shown in Figure 4, except the Taq DNA polymerase, were incubated for 3-5 min at 95° C. The

-42-

PCR was initiated by the addition of polymerase. Conditions were established and optimized using *Anabaena* DNA as template, in order to provide the best yield and lowest level of non-specific products for amplification of the target BC gene from *Anabaena* DNA. Amplification was for 45 cycles, each 1 min at 95°, 1 min at 42-46° and 2 min at 72° C. Both the reactions using *Anabaena* DNA and the single-stranded wheat cDNA as template yielded about 440-bp products. The wheat product was eluted from a gel and reamplified using the same primers. That product, also 440 bp, was cloned into the Invitrogen vector pCR1000 using their A/T tail method, and sequenced. The nucleotide sequence is shown in Figure 5.

In eukaryotic ACCs, the BCCP domain is located about 300 amino acids away from the end of the BC domain, on the C-terminal side. Therefore, it is possible to amplify the cDNA covering that interval using primers from the C-terminal end of the BC domain and the conserved MKM region of the BCCP. The BC primer was based on the wheat cDNA sequence obtained as described above. These primers, each with 6- or 8-base 5'-extensions, are shown in Figure 6B.

The MKM primer was first checked by determining whether it would amplify the *fabE* gene encoding BCCP from *Anabaena* DNA. This PCR was primed at the other end by using a primer based on the N-terminal amino acid sequence, determined on protein purified from *Anabaena* extracts by affinity chromatography, shown in Figure 6A. This amplification (using the conditions described above) worked, yielding the correct fragment of the *Anabaena fabE* gene, whose complete sequence is shown in Figure 7.

The PCR-amplified fragment of the *Anabaena fabE* gene was used to identify cosmids (three detected in a library of 1920) that contain the entire *fabE* gene and flanking DNA. A 4-kb XbaI fragment containing the gene was cloned into the vector Bluescript KS for sequencing. The two primers shown in Figure 6 were then used to amplify the intervening sequence in wheat cDNA. Again, the product of the first PCR was eluted and reamplified by another round of PCR, then cloned into the Invitrogen

-43-

vector pCRII. The complete 1.1 kb of the amplified DNA was sequenced, also shown in Figure 5.

5 The foregoing examples illustrate particular embodiments of the present invention. One of ordinary skill in the art will readily appreciate that changes, modifications and alterations to those embodiments can be made without departing from the true scope or spirit of the invention.

References

The references listed below and all references cited herein are incorporated herein by reference to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

1. J.R.Knowles. 1989. The mechanism of biotin-dependent enzymes. *Annu. Rev. Biochem.* 58: 195-221.
2. Alix, J.-H. 1989. A rapid procedure for cloning genes from libraries by complementation of *E. coli* defective mutants: application to the *fabE* region of the *E. coli* chromosome. *DNA* 8: 779-789.
3. Muramatsu, S., and T. Mizuno. 1989. Nucleotide sequence of the *fabE* gene and flanking regions containing a bent DNA sequence of *Escherichia coli*. *Nucleic Acids Res.* 17: 3982.
4. Li, S., and J. E. Cronan. 1992. The gene encoding the biotin carboxylase subunit of *Escherichia coli* acetyl-CoA carboxylase. *J. Biol. Chem.* 267: 855.
5. Lopez-Casillas, F., D. H. Bai, X. Luo, I. S. Kong, M. A. Hermodson, and K. H. Kim. 1988. Structure of the coding sequence and primary amino acid sequence of rat Acetyl-coenzyme A carboxylase. *Proc. Natl. Acad. Sci. USA* 85: 5784-5788.
6. Takai, T., C. Yokoyama, K. Wada, and T. Tanabe. 1988. Primary structure of chicken liver acetyl-coenzyme A

-45-

- carboxylase deduced from cDNA sequence. *J. Biol. Chem.* : 2651-2657.
- 6a. W. A. Feel, S. S. Chirala and S. J. Wakil 1992. Cloning of the yeast FAS3 gene and primary structure of yeast acetyl-CoA carboxylase. *Proc Natl Acad, Sci USA* 89: 4534-4538.
7. J.L.Harwood. 1988. Fatty acid metabolism. *Ann. Rev. Physiol. Plant Mol. Biol.* 39: 101-138.
8. Egin-Buhler, B., and J. Ebel. 1983. Improved purification and further characterization of ACC from culture cells of parsley. *Eur. J. Biochem.* 133: 335-339.
9. Wurtele, E.S. and Nikolau, B.J. 1990. *Arch. Biochem. Biophys.* 278: 179-186.
10. Slabas, A.R. and Hellyer, A. 1985. *Plant Sci.* 39: 177-182.
11. Samols, D., C. G. Thornton, V. L. Murtif, G. K. Kumar, F. C. Haase, and H. G. Wood. 1988. Evolutionary conservation among biotin enzymes. *J. Biol. Chem.* 263: 6461-6464.
12. H.K.Lichtenthaler. 1990. Mode of action of herbicides affecting acetyl-CoA carboxylase and fatty acid biosynthesis. *Z. Naturforsch.* 45c: 521-528.
13. I. Pecker, D. Chamovitz, H. Linden, G. Sandmann and J. Hirschberg. 1992. A single polypeptide catalyzing the conversion of phytoene to α -carotene is transcriptionally regulated during tomato fruit ripening. *Proc Natl Acad Sci USA* 89: 4962-4666.

-46-

14. G. K. Lamppa, G. Morelli and N-H Chua (1985). Structure and developmental regulation of a wheat gene encoding the major chlorophyll a/b-binding polypeptide. *Mol. Cell Biol.* 5: 1370-1378.
15. H. Haymerle, J. Herz, G. M. Bressan, R. Frank and K. K. Stanley (1986). Efficient construction of cDNA libraries in plasmid expression vectors using an adaptor strategy. *Nucl. Acids Res.* 14: 8615-8629.
16. V. Vasil, A. M. Castillo, M. E. Fromm and I. K. Vasil (1992). Herbicide-resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. *Biotechnology* 10: 667-674.
17. S. S. Golden, T. Brusslen and R. Haselkorn (1987), Genetic Enginerring of the Cyanobacterial Chromosome. *Methods Enzymology* 153: 215-231.

SEQUENCE LISTING:

(1) GENERAL INFORMATION:

- (i) Applicants: Robert Haselkorn and Piotr Gornicki
- (ii) Title of Invention: Cyanobacterial and Plant Acetyl-CoA Carboxylase
- (iii) Number of Sequences: 116
- (iv) Correspondence Address:
 - (A) Addressee: Arnold, White & Durkee
 - (B) Street: 321 North Clark Street
 - (C) City: Chicago
 - (D) State: Illinois
 - (E) Country: USA
 - (F) Zip: 60610
- (v) Computer Readable Form:
 - (A) Medium Type: Floppy Disk
 - (B) Computer: IBM PC Compatible
 - (C) Operating System: PC-DOS/MS-DOS
 - (D) Software: ASCII-DOS
- (vi) Current Application Data:
 - (A) Application Number: 07/956,700
 - (B) Filing Date: 10/21/92
 - (C) Classification: Unknown
- (vii) Attorney/Agent Information:
 - (A) Name: Thomas E. Northrup
 - (B) Registration Number: 33,268
 - (C) Reference/Docket Number: ARCD:058
- (viii) Telecommunication Information:
 - (A) Telephone: 1-312-744-0090
 - (B) Telefax: 1-312-755-4489

(2) INFORMATION FOR SEQ ID NO:1:

(i) Sequence characteristics:

(A) Length: 3065 base pairs
(B) Type: Nucleic acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule Type: Oligonucleotide

(xi) Sequence Description: SEQ ID NO:1:

AAGCTTTTAT ATTTTGCCAT TTCTAGAACT TAGCTGCATC GGCCCCAAGT ATTTTGTCAA 60
ATATGGCGAA AAGACTTCAT AAATCAAGGT TAAAGGTTGA CCGTGATGCC AAAACAGGTA 120
ATGGCGACCC CAGAAAGGCC CATCCACGCC AAAACCTAAT TGCAAGGCCT CTGAATTTCC 180
GTAATAAATA CCCCGCACAT CCCGATACAA CTCCGTGCCA AGACGAGCTA GACTTGCCCA 240
AATTGGTAAT GAACGGTTTT GCAAATACTC GTCTACATGG CTGGCTTCCC ACCATGAGGT 300
TGCATAGGCG AGTCGTTGGC CAGAGCGTGT ACGTAGCCAT ACCTGTCGCC GCAGTCTTGG 360
CGCTGGAACA GATTGGATTA AATCCGGCGC ACTATCTAAA TCCAAACCAA TCAATGACAT 420
ATCAATGACA TCGACTTCTG TTGGCTCACC AGTAAGTAAT TCTAAATGCC TTGTGGGTGA 480
GCCATCACCT AAGAGTAGTA GTTGCCACGC TGGAGCCAGC TGAGTGTGAG GCAAACATATG 540
TTAATTACT TCTTCCCCAC CTTGCCAAT AGGAGTGAGG CGATGCCATC CGGCTGGCAG 600
TGTTGAGTTG TTGCTTGGAG TAAAAGTGGC AGTCAATGTT CTTTACAAA GTTCACCTAT 660
TTATATCAAA GCATAAAAAA TTAATTAGTT GTCAGTTGTC ATTGGTTATT CTTCTTTGCT 720
CCCCCTGCCC CTTACTTCCC TCCTCTGCCC AATAATTAGA AAGGTCAGGA GTCAAAAAC 780
TATCACTTTT GACCACTGAC CTTTCACAAT TGAATATAGT CACTAAAAAA TCGCGATGGC 840
GAGACTCGAA CTCGCAAGGC AAAGCCACAC GCACCTCAAG CGTGCGCGTA TACCAATTCC 900
GCCACATCCG CACGGGTTGT ACAAGAAGAT ATACTAGCAC AAAAAAATTG CATAAAACAA 960

GGTAAACTA TATTTGCCAA ACTTTATGGA AAATTTATCT TGCTAAATAT ACAAATTTCC 1020
CGAAGAGGAT ACGAGACTAA CAGAAATGTA GTATCGCCAC AAGTGATATT AAAGGGGGTA 1080
TGGGGGTTTT CTTCCCTTAC ACCCTTAAAC CCTCACACCC CACCTCCATG AAAAATCTTG 1140
TTGGTAAGTC CGTTTCCTGC AATTTATTTA AAGATGAGCC TGGGGTATCT CCTGTCATAA 1200
TTTGAGATGA AGCGATGCCT AAGGCGGCTA CGCTACGCGC TAAAAGCAAC TTGGATGGGA 1260
GACAATTTCT ATCTGCTGGT ACTGATACTG ATATCGAAAA CTAGAAAATG AAGTTTGACA 1320
AAATATTAAT TGCCAATCGG GGAGAAATAG CGCTGCGCAT TCTCCGCGCC TGTGAGGAAA 1380
TGGGGATTGC GACGATCGCA GTTCATTCTA CTGTTGACCG GAATGCTCTT CATGTCCAAC 1440
TTGCTGACGA AGCGGTTTGT ATTGGCGAAC CTGCTAGCGC TAAAAGTTAT TTGAATATTC 1500
CCAATATTAT TGCTGCGGCT TTAACGCGCA ATGCCAGTGC TATTCATCCT GGGTATGGCT 1560
TTTTATCTGA AAATGCCAAA TTTGCGGAAA TCTGTGCTGA CCATCACATT GCATTCATTG 1620
GCCCCACCCC AGAAGCTATC CGCCTCATGG GGGACAAATC CACTGCCAAG GAAACCATGC 1680
AAAAAGCTGG TGTACCGACA GTACCGGGTA GTGAAGGTTT GGTAGAGACA GAGCAAGAAG 1740
GATTAGAACT GGCAGAAAGAT ATTGGCTACC CAGTGATGAT CAAAGCCACG GCTGGTGGTG 1800
GCGGCCGGGG TATGCGACTG GTGCGATCGC CAGATGAATT TGTCAAACCTG TTCTTAGCCG 1860
CCCAAGGTGA AGCTGGTGCA GCCTTTGGTA ATGCTGGCGT TTATATAGAA AAATTTATTG 1920
AACGTCCGCG CCACATTGAA TTTCAAATTT TGGCTGATAA TTACGGCAAT GTGATTCACT 1980
TGGGTGAGAG GGATTGCTCA ATTCAGCGTC GTAACCAAAA GTTACTAGAA GAAGCCCCCA 2040
GCCCAGCCTT GGAATCAGAC CTAAGGGAAA AAATGGGACA AGCGGCGGTG AAAGCGGCTC 2100
AGTTTATCAA TTACGCCGGG GCAGGTACTA TCGAGTTTTT GCTAGATAGA TCCGGTCAGT 2160
TTTACTTTAT GGAGATGAAC ACCCGGATTC AAGTAGAACA TCCCCTAACT GAGATGGTTA 2220

CTGGAGTGGA TTTATTGGTT GAGCAAATCA GAATTGCCCA AGGGGAAAGA CTTAGACTAA 2280
CTCAAGACCA AGTAGTTTAA CGCGGTCATG CGATCGAATG TCGCATCAAT GCCGAAGACC 2340
CAGACCACGA TTTCCGCCCC GCACCCGGAC GCATTAGCGG TTATCTTCCC CCTGGCGGGC 2400
CTGGCGTGCG GATTGACTCC CACGTTTACA CGGATTACCA AATCCGCCC TACTACGATT 2460
CCTTAATTGG TAAATTGATC GTTTGGGGCC CTGATCGCGC TACTGCTATT AACC GCATGA 2520
AACGCGCCCT CAGGGAATGC GCCATCACTG GATTACCTAC AACCATTGGG TTTCATCAA 2580
GAATTATGGA AAATCCCCAA TTTTACAAG GTAATGTGTC TACTAGTTTT GTGCAGGAGA 2640
TGAATAAATA GGGTAATGGG TAATGGGTAA TGGGTAATAG AGTTTCAATC ACCAATTACC 2700
AATCCCTAA CTCATCCGTG CCAACATCGT CAGTAATCCT TGCTGGCCTA GAAGAACTTC 2760
TCGCAACAGG CTAAAAATAC CAACACACAC AATGGGGGTG ATATCAACAC CACCTATTGG 2820
TGGGATGATT TTTCGCAAGG GAATGAGAAA TGGTTCAGTC GGCCAAGCAA TTAAGTTGAA 2880
GGGCAAACGG TTCAGATCGA CTTGCGGATA CCAGGTCAGA ATGATACGGA AAATAAACAG 2940
AAATGTCATC ACTCCCAATA CAGGGCCAAG AATCCAAACG CTCAGGTTAA CACCAGTCAT 3000
CGATCTAAGC TACTATTTTG TGAATTTACA AAAA ACTGCA AGCAAAAGCT GAAAATTTTA 3060
AGCTT 3065

(i) **Sequence characteristics:**

(A) Length: 32 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:2:

Asp Glu Ala Met Pro Lys Ala Ala Thr Leu Arg Ala Lys Ser Asn Leu
5 10 15

Asp Gly Arg Gln Phe Leu Ser Ala Gly Thr Asp Thr Asp Ile Glu Asn
20 25 30

(i) Sequence characteristics:

(A) Length: 427 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:3:

Lys	Met	Lys	Phe	Asp	Lys	Ile	Leu	Ile	Ala	Asn	Arg	Gly	Glu	Ile	Ala
			5						10					15	
Leu	Arg	Ile	Leu	Arg	Ala	Cys	Glu	Glu	Met	Gly	Ile	Ala	Thr	Ile	Ala
			20					25					30		
Val	His	Ser	Thr	Val	Asp	Arg	Asn	Ala	Leu	His	Val	Gln	Leu	Ala	Asp
		35					40					45			
Glu	Ala	Val	Cys	Ile	Gly	Glu	Pro	Ala	Ser	Ala	Lys	Ser	Tyr	Leu	Asn
	50					55					60				
Ile	Pro	Asn	Ile	Ile	Ala	Ala	Ala	Leu	Thr	Arg	Asn	Ala	Ser	Ala	Ile
65					70					75					80
His	Pro	Gly	Tyr	Gly	Phe	Leu	Ser	Glu	Asn	Ala	Lys	Phe	Ala	Glu	Ile
				85					90					95	
Cys	Ala	Asp	His	His	Ile	Ala	Phe	Ile	Gly	Pro	Thr	Pro	Glu	Ala	Ile
			100					105					110		
Arg	Leu	Met	Gly	Asp	Lys	Ser	Thr	Ala	Lys	Glu	Thr	Met	Gln	Lys	Ala
		115					120					125			
Gly	Val	Pro	Thr	Val	Pro	Gly	Ser	Glu	Gly	Leu	Val	Glu	Thr	Glu	Gln
	130					135					140				

52

Glu Gly Leu Glu Leu Ala Lys Asp Ile Gly Tyr Pro Val Met Ile Lys
 145 150 155 160
 Ala Thr Ala Gly Gly Gly Gly Arg Gly Met Arg Leu Val Arg Ser Pro
 165 170 175
 Asp Glu Phe Val Lys Leu Phe Leu Ala Ala Gln Gly Glu Ala Gly Ala
 180 185 190
 Ala Phe Gly Asn Ala Gly Val Tyr Ile Glu Lys Phe Ile Glu Arg Pro
 195 200 205
 Arg His Ile Glu Phe Gln Ile Leu Ala Asp Asn Tyr Gly Asn Val Ile
 210 215 220
 His Leu Glu Arg Asp Cys Ser Ile Gln Arg Arg Asn Gln Lys Leu Leu
 225 230 235 240
 Glu Glu Ala Pro Ser Pro Ala Leu Asp Ser Asp Leu Arg Glu Lys Met
 245 250 255
 Gly Gln Ala Ala Val Lys Ala Ala Gln Phe Ile Asn Tyr Ala Gly Ala
 260 265 270
 Gly Thr Ile Glu Phe Leu Leu Asp Arg Ser Gly Gln Phe Gly Val Asp
 275 280 285
 Leu Leu Val Glu Gln Ile Arg Ile Ala Gln Gly Glu Arg Leu Arg Leu
 290 295 300
 Thr Gln Asp Gln Val Val Leu Arg Gly His Ala Ile Glu Cys Arg Ile
 305 310 315 320
 Asn Ala Glu Asp Pro Asp His Asp Phe Arg Pro Ala Pro Gly Arg Ile
 325 330 335
 Ser Gly Tyr Leu Pro Pro Gly Gly Pro Gly Val Arg Ile Asp Ser His
 340 345 350
 Val Tyr Thr Asp Tyr Gln Ile Pro Pro Tyr Tyr Asp Ser Leu Ile Gly
 355 360 365
 Lys Leu Ile Val Trp Gly Pro Asp Arg Ala Thr Ala Ile Asn Arg Met
 370 375 380
 Lys Arg Ala Leu Arg Glu Cys Ala Ile Thr Gly Leu Pro Thr Thr Ile
 385 390 395 400
 Gly Phe His Gln Arg Ile Met Glu Asn Pro Gln Phe Leu Gln Gly Asn
 405 410 415
 Val Ser Thr Ser Phe Val Gln Glu Met Asn Lys
 420 425

(i) Sequence characteristics:

- (A) Length: 36 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:4:

[illegible]

(i) Sequence characteristics:

- (A) Length: 1342 base pairs
(B) Type: Nucleic acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Oligonucleotide

(xi) Sequence Description: SEQ ID NO:5:

ATGCGTTTTCA	ACAAGATCCT	GATCGCCAAT	CGCGGCGAAA	TCGCCCTGCG	CATTCTCCGC	60
ACTTGTCAAG	AACTCGGGAT	CGGCACGATC	GCCGTTCACT	CCACTGTGGA	TCGCAACGCG	120
CTCCATGTGC	AGTTAGCGGA	CGAAGCGGTC	TGTATTGGCG	AAGCGGCCAG	CAGCAAAAGC	180
TATCTCAATA	TCCCCAACAT	CATTGCGGCG	GCCCTGACCC	CTAATGCCAG	CGCCATTAC	240
CCCGGCTATG	GCTTCTTGGC	GGAGAATGCC	CGCTTTGCAG	AAATCTGCGC	CGATCACCAT	300
CTCACCTTTA	TTGGCCCCAG	CCCCGATTCTG	ATTCGAGCCA	TGGGCGATAA	ATCCACCCT	360
AAGGAAACAA	TGCAGCGGGT	CGGCGTTCCG	ACGATTCCGG	GCAGTGACGG	TCTGCTGACG	400
GATGTTGATT	CGGCTGCCAA	AGTTGCTGCC	GAGATCGGCT	ATCCCGTCAT	GATCAAAGCG	460
ACGGCGGGGG	GCGGTGGTCG	CGGTATGCGG	CTGGTGCGTG	ACCCTGCAGA	TCTGGAAAAA	520
CTGTTCTTGG	CTGCCCAAGG	AGAAGCCGAG	GCAGCTTTTG	GGAATCCAGG	ACTGTATCTC	580
GAAAAATTTA	TCGATCGCCC	ACGCCACGTT	GAATTTCAGA	TCTTGGCCGA	TGCCTACGGC	640
AATGTAGTGC	ATCTAGGCGA	GCGCGATTGC	TCCATTCAAC	GTCGTACCA	AAAGCTGCTC	700
GAAGAAGCCC	CCAGTCCGGC	GCTATCGGCA	GACCTGCGGC	AGAAAATGGG	CGATGCCGCC	760

54

GTCAAAGTCG CTCAAGCGAT CGGCTACATC GGTGCCGGCA CCGTGGAGTT TCTGGTCGAT 820
 GCGACCGGCA ACTTCTACTT CATGGAGATG AATACCCGCA TCCAAGTCGA GCATCCAGTC 900
 ACAGAAATGA TTACGGGACT GGACTTGATT GCGGAGCAGA TTCGGATTGC CCAAGGCGAA 960
 GCGCTGCGCT TCCGGCAAGC CGATATTCAA CTGCGCGGCC ATGCGATCGA ATGCCGTATC 1020
 AATGCGGAAG ATCCGGAATA CAATTTCCGG CCGAATCCTG GCCGCATTAC AGGCTATTTA 1080
 CCGCCCCGCG GCCCCGCGT TCGTGTCGAT TCCCATGTTT ATACCGACTA CGAAATTCCG 1140
 CCCTATTACG ATTCGCTGAT TGGCAAATTG ATTGTCTGGG GTGCAACACG GGAAGAGGCG 1200
 ATCGCGCGGA TGCAGCGTGC TCTGCGGGAA TCGCCCATCA CCGGCTTGCC GACGACCCCT 1260
 AGTTTCCATC AGCTGATGTT GCAGATGCCT GAGTTCCTGC GCGGGGAACT CTATACCAAC 1300
 TTTGTTGAGC AGGTGATGCT ACCTCGGATC CTCAAGTCCT AG 1342

(2) INFORMATION FOR SEQ ID NO:6:

(i) Sequence characteristics:

(A) Length: 453 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:6:

Met Arg Phe Asn Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Leu
 5 10 15
 Arg Ile Leu Arg Thr Cys Glu Glu Leu Gly Ile Gly Thr Ile Ala Val
 20 25 30
 His Ser Thr Val Asp Arg Asn Ala Leu His Val Gln Leu Ala Asp Glu
 35 40 45
 Ala Val Cys Ile Gly Glu Ala Ala Ser Ser Lys Ser Tyr Leu Asn Ile
 50 55 60
 Pro Asn Ile Ile Ala Ala Ala Leu Thr Arg Asn Ala Ser Ala Ile His
 65 70 75 80
 Pro Gly Tyr Gly Phe Leu Ala Glu Asn Ala Arg Phe Ala Glu Ile Cys
 85 90 95
 Ala Asp His His Leu Thr Phe Ile Gly Pro Ser Pro Asp Ser Ile Arg
 100 105 110
 Ala Met Gly Asp Lys Ser Thr Ala Lys Glu Thr Met Gln Arg Val Gly
 115 120 125
 Val Pro Thr Ile Pro Gly Ser Asp Gly Leu Leu Thr Asp Val Asp Ser
 130 135 140

55

Ala Ala Lys Val Ala Ala Glu Ile Gly Tyr Pro Val Met Ile Lys Ala
 145 150 155 160
 Thr Ala Gly Gly Gly Gly Arg Gly Met Arg Leu Val Arg Glu Pro Ala
 165 170 175
 Asp Leu Glu Lys Leu Phe Leu Ala Ala Gln Gly Glu Ala Glu Ala Ala
 180 185 190
 Phe Gly Asn Pro Gly Leu Tyr Leu Glu Lys Phe Ile Asp Arg Pro Arg
 195 200 205
 His Val Glu Phe Gln Ile Leu Ala Asp Ala Tyr Gly Asn Val Val Glu
 210 215 220
 Leu Gly Glu Arg Asp Cys Ser Ile Gln Arg Arg His Gln Lys Leu Leu
 225 230 235 240
 Glu Glu Ala Pro Ser Pro Ala Leu Ser Ala Asp Leu Arg Gln Lys Met
 245 250 255
 Gly Asp Ala Ala Val Lys Val Ala Gln Ala Ile Gly Tyr Ile Gly Ala
 260 265 270
 Gly Thr Val Glu Phe Leu Val Asp Ala Thr Gly Asn Phe Tyr Phe Met
 275 280 285
 Glu Met Asn Thr Arg Ile Gln Val Glu His Pro Val Thr Glu Met Ile
 290 295 300
 Thr Gly Leu Asp Leu Ile Ala Glu Gln Ile Arg Ile Ala Gln Gly Glu
 305 310 315 320
 Ala Leu Arg Phe Arg Gln Ala Asp Ile Gln Leu Arg Gly His Ala Ile
 325 330 335
 Glu Cys Arg Ile Asn Ala Glu Asp Pro Glu Tyr Asn Phe Arg Pro Asn
 340 345 350
 Pro Gly Arg Ile Thr Gly Tyr Leu Pro Pro Gly Gly Pro Gly Val Arg
 355 360 365
 Val Asp Ser His Val Tyr Thr Asp Tyr Glu Ile Pro Pro Tyr Tyr Asp
 370 375 380
 Ser Leu Ile Gly Lys Leu Ile Val Trp Gly Ala Thr Arg Glu Glu Ala
 385 390 395 400
 Ile Ala Arg Met Gln Arg Ala Leu Arg Glu Gly Ala Ile Thr Gly Leu
 405 410 415
 Pro Thr Thr Leu Ser Phe His Gln Leu Met Leu Gln Met Pro Glu Phe
 420 425 430
 Leu Arg Gly Glu Leu Tyr Thr Asn Phe Val Glu Gln Val Met Leu Pro
 435 440 445
 Arg Ile Leu Lys Ser
 450

(1) Sequence characteristics:

(ii) Molecule type: Peptide

```
Met Asp Glu Pro Ser Pro Leu Ala Lys Thr Leu Glu Leu Asn Gln His  
5 10 15  
Ser Arg Phe Ile Ile Gly Ser Val Ser Glu Asp Asn Ser Glu Asp Glu  
20 25 30  
Ile Ser
```

(i) Sequence characteristics:

(ii) Molecule type: Peptide

Asn	Leu	Val	Lys	Leu	Asp	Leu	Glu	Glu	Lys	Glu	Gly	Ser	Leu	Ser	Pro
				5					10					15	
Ala	Ser	Val	Ser	Ser	Asp	Thr	Leu	Ser	Asp	Leu	Gly	Ile	Ser	Ala	Leu
			20					25					30		
Gln	Asp	Gly	Leu	Ala	Phe	His	Met	Arg	Ser	Ser	Met	Ser	Gly	Leu	His
		35					40					45			
Leu	Val	Lys	Gln	Gly	Arg	Asp	Arg	Lys	Lys	Ile	Asp	Ser	Gln	Arg	Asp
	50					55					60				
Phe	Thr	Val	Ala	Ser	Pro	Ala	Glu	Phe	Val	Thr	Arg	Phe	Gly	Gly	Asn
65					70					75					80
Lys	Val	Ile	Glu	Lys	Val	Leu	Ile	Ala	Asn	Asn	Gly	Ile	Ala	Ala	Val
				85					90					95	
Lys	Cys	Met	Arg	Ser	Ile	Arg	Arg	Trp	Ser	Tyr	Glu	Met	Phe	Arg	Asn
			100					105					110		
Glu	Arg	Ala	Ile	Arg	Phe	Val	Val	Met	Val	Thr	Pro	Glu	Asp	Leu	Lys
		115					120					125			

57

Ala Asn Ala Glu Tyr Ile Lys Met Ala Asp His Tyr Val Pro Val Pro
 130 135 140
 Gly Gly Ala Asn Asn Asn Asn Tyr Ala Asn Val Glu Leu Ile Leu Asp
 145 150 155 160
 Ile Ala Lys Arg Ile Pro Val Gln Ala Val Trp Ala Gly Trp Gly His
 165 170 175
 Ala Ser Glu Asn Pro Lys Leu Pro Glu Leu Leu
 180 185

(2) INFORMATION FOR SEQ ID NO:9:

(i) Sequence characteristics:

(A) Length: 122 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:9:

Leu Lys Asn Gly Ile Ala Phe Met Gly Pro Pro Ser Gln Ala Met Trp
 5 10 15
 Ala Leu Gly Asp Lys Ile Ala Ser Ser Ile Val Ala Gln Thr Ala Gly
 20 25 30
 Ile Pro Thr Leu Pro Trp Ser Gly Ser Gly Leu Arg Val Asp Trp Gln
 35 40 45
 Glu Asn Asp Phe Ser Lys Arg Ile Leu Asn Val Pro Gln Asp Leu Tyr
 40 55 60
 Glu Lys Gly Tyr Val Lys Asp Val Asp Asp Gly Leu Lys Ala Ala Glu
 65 70 75 80
 Glu Val Gly Tyr Pro Val Met Ile Lys Ala Ser Glu Gly Gly Gly Gly
 85 90 95
 Lys Gly Ile Arg Lys Val Asn Asn Ala Asp Asp Phe Pro Asn Leu Phe
 100 105 110
 Arg Gln Val Gln Ala Glu Val Pro Gly Ser
 115 120

58

(2) INFORMATION FOR SEQ ID NO:10:

(i) Sequence characteristics:

(A) Length: 86 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:10:

Pro Ile Phe Val Met Arg Leu Ala Lys Gln Ser Arg His Leu Glu Val
 5 10 15
 Gln Ile Leu Ala Asp Gln Tyr Gly Asn Ala Ile Ser Leu Phe Gly Arg
 20 25 30
 Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile Ile Glu Glu Ala Pro
 35 40 45
 Ala Ala Ile Ala Thr Pro Ala Val Phe Glu His Met Glu Gln Cys Ala
 50 55 60
 Val Lys Leu Ala Lys Met Val Gly Tyr Val Ser Ala Gly Thr Val Glu
 65 70 75 80
 Tyr Leu Tyr Ser Gln Asp
 85

(2) INFORMATION FOR SEQ ID NO:11:

(i) Sequence characteristics:

(A) Length: 70 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:11:

Gly Ser Phe Tyr Phe Leu Glu Leu Asn Pro Arg Leu Gln Val Glu His
 5 10 15
 Pro Cys Thr Glu Met Val Ala Asp Val Asn Leu Pro Ala Ala Gln Leu
 20 25 30
 Gln Ile Ala Met Gly Ile Pro Leu Phe Arg Ile Lys Asp Ile Arg Met
 35 40 45
 Met Tyr Gly Val Ser Pro Trp Gly Asp Ala Pro Ile Asp Phe Glu Asn
 50 55 60
 Ser Ala His Val Pro Cys
 70

(2) INFORMATION FOR SEQ ID NO:12:

(i) Sequence characteristics:

(A) Length: 20 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:12:

Pro Arg Gly His Val Ile Ala Ala Arg Ile Thr Ser Glu Asn Pro Asp
 5 10 15

Glu Gly Phe Lys
 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:13:

Pro Ser Ser Gly Thr Val Gln Glu Leu Asn Phe Arg Ser Asn Lys Asn
 5 10 15

Val Trp Gly Tyr Phe
 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) Sequence characteristics:

(A) Length: 122 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:14:

Ser Val Ala Ala Ala Gly Gly Leu His Glu Phe Ala Asp Ser Gln Phe
 5 10 15

Gly His Cys Phe Ser Trp Gly Glu Asn Arg Glu Glu Ala Ile Ser Asn
 20 25 30

60

Met Val Val Ala Leu Lys Glu Leu Ser Ile Arg Gly Asp Phe Arg Thr
 35 40 45
 Thr Val Glu Tyr Leu Ile Lys Leu Leu Glu Thr Glu Ser Phe Gln Leu
 50 55 60
 Asn Arg Ile Asp Thr Gly Trp Leu Asp Arg Leu Ile Ala Glu Lys Val
 65 70 75 80
 Gln Ala Glu Arg Pro Asp Thr Met Leu Gly Val Val Cys Gly Ala Leu
 85 90 95
 His Val Ala Asp Val Asn Leu Arg Asn Ser Ile Ser Asn Phe Leu His
 100 105 110
 Ser Leu Glu Arg Gly Gln Val Leu Pro Ala
 115 120

(2) INFORMATION FOR SEQ ID NO:15:

(i) Sequence characteristics:

(A) Length: 190 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:15:

His Thr Leu Leu Asn Thr Val Asp Val Glu Leu Ile Tyr Glu Gly Ile
 5 10 15
 Lys Tyr Val Leu Lys Val Thr Arg Gln Ser Pro Asn Ser Tyr Val Val
 20 25 30
 Ile Met Asn Gly Ser Cys Val Glu Val Asp Val His Arg Leu Ser Asp
 35 40 45
 Gly Gly Leu Leu Leu Ser Tyr Asp Gly Ser Ser Tyr Thr Thr Tyr Met
 50 55 60
 Lys Glu Glu Val Asp Arg Tyr Arg Ile Thr Ile Gly Asn Lys Thr Cys
 65 70 75 80
 Val Phe Glu Lys Glu Asn Asp Pro Ser Val Met Arg Ser Pro Ser Ala
 85 90 95
 Gly Lys Leu Ile Gln Tyr Ile Val Glu Asp Gly Gly His Val Phe Ala
 100 105 110
 Gly Gln Cys Tyr Ala Glu Ile Glu Val Met Lys Met Val Met Thr Leu
 115 120 125
 Thr Ala Val Glu Ser Gly Cys Ile His Tyr Val Lys Arg Pro Gly Ala
 130 135 140
 Ala Leu Asp Pro Gly Cys Val Ile Ala Lys Met Gln Leu Asp Asn Pro
 145 150 155 160

61

Ser Lys Val Gln Gln Ala Glu Leu His Thr Gly Ser Leu Pro Gln Ile
 165 170 175
 Gln Ser Thr Ala Leu Arg Gly Glu Lys Leu His Arg Ile Phe
 180 185 190

(2) INFORMATION FOR SEQ ID NO:16:

(i) Sequence characteristics:

(A) Length: 37 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:16:

Val Met Ile Lys Ala Ser Trp Gly Gly Gly Gly Lys Gly Ile Arg Lys
 5 10 15
 Val His Asn Asp Asp Glu Val Arg Ala Leu Phe Lys Gln Val Gln Gly
 20 25 30
 Glu Val Pro Gly Ser
 35

(2) INFORMATION FOR SEQ ID NO:17:

(i) Sequence characteristics:

(A) Length: 187 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:17:

Pro Ile Phe Ile Met Lys Val Ala Ser Gln Ser Arg His Leu Glu Val
 5 10 15
 Gln Leu Leu Cys Asp Lys His Gly Asn Val Ala Ala Leu His Ser Arg
 20 25 30
 Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile Ile Glu Glu Gly Pro
 35 40 45
 Ile Thr Val Ala Pro Pro Glu Thr Ile Lys Glu Leu Glu Gln Ala Ala
 50 55 60
 Arg Arg Leu Ala Lys Cys Val Gln Tyr Gln Gly Ala Ala Thr Val Glu
 65 70 75 80
 Tyr Leu Tyr Ser Met Glu Thr Gly Glu Tyr Tyr Phe Leu Glu Leu Asn
 85 90 95

62

Pro Arg Leu Gln Val Glu His Pro Val Thr Glu Trp Ile Ala Glu Ile
 100 105 110

Asn Leu Pro Ala Ser Gln Val Val Val Gly Met Gly Ile Pro Leu Tyr
 115 120 125

Asn Ile Pro Glu Ile Arg Arg Phe Tyr Gly Ile Glu His Gly Gly Gly
 130 135 140

Tyr His Ala Trp Lys Glu Ile Ser Ala Val Ala Thr Lys Phe Asp Leu
 145 150 155 160

Asp Lys Ala Gln Ser Val Lys Pro Lys Gly His Cys Val Ala Val Arg
 165 170 175

Val Thr Ser Glu Asp Pro Asp Asp Gly Phe Lys
 180 185

(2) INFORMATION FOR SEQ ID NO:18:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:18:

Pro Thr Ser Gly Arg Val Glu Glu Leu Asn Phe Lys Ser Lys Pro Asn
 5 10 15

Val Trp Ala Tyr Phe
 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) Sequence characteristics:

(A) Length: 122 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:19:

Ser Val Lys Ser Gly Gly Ala Ile His Glu Phe Ser Asp Ser Gln Phe
 5 10 15

Gly His Val Phe Ala Phe Gly Glu Ser Arg Ser Leu Ala Ile Ala Asn
 20 25 30

Met Val Leu Gly Leu Lys Glu Ile Gln Ile Arg Gly Glu Ile Arg Thr
 35 40 45

63

Asn Val Asp Tyr Thr Val Asp Leu Leu Asn Ala Ala Glu Tyr Arg Glu
 50 55 60
 Asn Met Ile His Thr Gly Trp Leu Asp Ser Arg Ile Ala Met Arg Val
 65 70 75 80
 Arg Ala Glu Arg Pro Pro Trp Tyr Leu Ser Val Val Gly Gly Ala Leu
 85 90 95
 Tyr Glu Ala Ser Ser Arg Ser Ser Ser Val Val Thr Asp Tyr Val Gly
 100 105 110
 Tyr Leu Ser Lys Gly Gln Ile Pro Pro Lys
 110 120

(2) INFORMATION FOR SEQ ID NO:20:

(i) Sequence characteristics:

(A) Length: 124 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:20:

His Ile Ser Leu Val Asn Leu Thr Val Thr Leu Asn Ile Asp Gly Ser
 5 10 15
 Lys Tyr Thr Ile Glu Thr Val Arg Gly Gly Pro Arg Ser Tyr Lys Leu
 20 25 30
 Arg Ile Asn Glu Ser Glu Val Glu Ala Glu Ile His Phe Leu Arg Asp
 35 40 45
 Gly Gly Leu Leu Met Gln Leu Asp Gly Asn Ser His Val Ile Tyr Ala
 50 55 60
 Glu Thr Glu Ala Ala Gly Thr Arg Leu Leu Ile Asn Gly Arg Thr Cys
 65 70 75 80
 Leu Leu Gln Lys Glu His Asp Pro Ser Arg Leu Leu Ala Asp Thr Pro
 85 90 95
 Cys Lys Leu Leu Arg Phe Leu Val Ala Asp Gly Ser His Val Val Ala
 100 105 110
 Asp Thr Pro Tyr Ala Glu Val Glu Ala Met Lys Met
 115 120

(2) INFORMATION FOR SEQ ID NO:21:

(i) Sequence characteristics:

(A) Length: 222 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:21:

Met	Glu	Glu	Ser	Ser	Gln	Pro	Ala	Lys	Pro	Leu	Glu	Met	Asn	Pro	His	
				5					10					15		
Ser	Arg	Phe	Ile	Ile	Gly	Ser	Val	Ser	Glu	Asp	Asn	Ser	Glu	Asp	Glu	
				20					25					30		
Thr	Ser	Ser	Leu	Val	Lys	Leu	Asp	Leu	Leu	Glu	Glu	Lys	Glu	Arg	Ser	
				35					40					45		
Leu	Ser	Pro	Val	Ser	Val	Cys	Ser	Asp	Ser	Leu	Ser	Asp	Leu	Gly	Leu	
				50					55					60		
Pro	Ser	Ala	Gln	Asp	Gly	Leu	Ala	Asn	His	Met	Arg	Pro	Ser	Met	Ser	
				65					70					75		
Gly	Leu	His	Leu	Val	Lys	Gln	Gly	Arg	Asp	Arg	Lys	Lys	Val	Asp	Val	
				85					90					95		
Gln	Arg	Asp	Phe	Thr	Val	Ala	Ser	Pro	Ala	Glu	Phe	Val	Thr	Arg	Phe	
				100					105					110		
Gly	Gly	Asn	Arg	Val	Ile	Glu	Lys	Val	Leu	Ile	Ala	Asn	Asn	Gly	Ile	
				115					120					125		
Ala	Ala	Val	Lys	Cys	Met	Arg	Ser	Ile	Arg	Arg	Trp	Ser	Tyr	Glu	Met	
				130					135					140		
Phe	Arg	Asn	Glu	Arg	Ala	Ile	Arg	Phe	Val	Val	Met	Val	Thr	Pro	Glu	
				145					150					155		
Asp	Leu	Lys	Ala	Asn	Ala	Glu	Tyr	Ile	Lys	Met	Ala	Asp	His	Tyr	Val	
				165					170					175		
Pro	Val	Pro	Gly	Gly	Pro	Asn	Asn	Asn	Asn	Tyr	Ala	Asn	Val	Glu	Leu	
				180					185					190		
Ile	Leu	Asp	Ile	Ala	Lys	Arg	Ile	Pro	Val	Gln	Ala	Val	Trp	Ala	Gly	
				195					200					205		
Trp	Gly	His	Ala	Ser	Glu	Asn	Pro	Lys	Leu	Pro	Glu	Leu	Leu			
				210					215					220		

65

(2) INFORMATION FOR SEQ ID NO:22:

(i) Sequence characteristics:

(A) Length: 122 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:22:

His Lys Asn Gly Ile Ala Phe Met Gly Pro Pro Ser Gln Ala Met Trp
 5 10 15
 Ala Leu Gly Asp Lys Ile Ala Ser Ser Ile Val Ala Gln Thr Ala Gly
 20 25 30
 Ile Pro Thr Leu Pro Trp Asn Gly Ser Gly Leu Arg Val Asp Trp Gln
 35 40 45
 Glu Asn Asp Leu Gln Lys Arg Ile Leu Asn Val Pro Gln Glu Leu Tyr
 50 55 60
 Glu Lys Gly Tyr Val Lys Asp Ala Asp Asp Gly Leu Arg Ala Ala Glu
 65 70 75 80
 Glu Val Gly Tyr Pro Val Met Ile Lys Ala Ser Glu Gly Gly Gly Gly
 85 90 95
 Lys Gly Ile Arg Lys Val Asn Asn Ala Asp Asp Phe Pro Asn Leu Phe
 100 105 110
 Arg Gln Val Gln Ala Glu Val Pro Gly Ser
 115 120

(2) INFORMATION FOR SEQ ID NO:23:

(i) Sequence characteristics:

(A) Length: 95 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:23:

Pro Ile Phe Val Met Arg Leu Ala Lys Gln Ser Arg His Leu Glu Val
 5 10 15
 Gln Ile Leu Ala Asp Gln Tyr Gly Asn Ala Ile Ser Leu Phe Gly Arg
 20 25 30
 Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile Ile Glu Ala Gly
 35 40 45

66

Leu Arg Ala Ala Glu Glu Val Gly Tyr Pro Val Met Ile Lys Ala Ser
 50 55 60

Glu Gly Gly Gly Gly Lys Gly Ile Arg Lys Val Asn Asn Ala Asp Asp
 65 70 75 80

Phe Pro Asn Leu Phe Arg Gln Val Gln Ala Glu Val Pro Gly Ser
 80 90 95

(2) INFORMATION FOR SEQ ID NO:24:**(i) Sequence characteristics:**

(A) Length: 86 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide**(xi) Sequence Description: SEQ ID NO:24:**

Pro Ile Phe Val Met Arg Leu Ala Lys Gln Ser Arg His Leu Glu Val
 5 10 15

Gln Ile Leu Ala Asp Gln Tyr Gly Asn Ala Ile Ser Leu Phe Gly Arg
 20 25 30

Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile Ile Glu Glu Ala Pro
 35 40 45

Ala Ser Ile Ala Thr Ser Val Val Phe Glu His Met Glu Gln Cys Ala
 50 55 60

Val Lys Leu Ala Lys Met Val Gly Tyr Val Ser Ala Gly Thr Val Glu
 65 70 75 80

Tyr Leu Tyr Ser Gln Asp
 85

(2) INFORMATION FOR SEQ ID NO:25:**(i) Sequence characteristics:**

(A) Length: 70 amino acids
 (B) Type: Amino acids
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide**(xi) Sequence Description: SEQ ID NO:25:**

Gly Ser Phe Tyr Phe Leu Glu Leu Asn Pro Arg Leu Gln Val Glu His
 5 10 15

Pro Cys Thr Glu Met Val Ala Asp Val Asn Leu Pro Ala Ala Gln Leu
 20 25 30

67

Gln Ile Ala Met Gly Ile Pro Leu His Arg Ile Lys Asp Ile Arg Val
 35 40 45
 Met Tyr Gly Val Ser Pro Trp Gly Asp Gly Ser Ile Asp Phe Glu Asn
 50 35 60
 Ser Ala His Val Pro Cys
 65 70

(2) INFORMATION FOR SEQ ID NO:26:

(i) Sequence characteristics:

(A) Length: 20 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:26:

Pro Arg Gly His Val Ile Ala Ala Arg Ile Thr Ser Glu Asn Pro Asp
 5 10 15
 Glu Gly Phe Lys
 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:27:

Pro Ser Ser Gly Thr Val Gln Glu Leu Asn Phe Arg Ser Asn Lys Asn
 5 10 15
 Val Trp Gly Tyr Phe
 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) Sequence characteristics:

(A) Length: 122 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:28:

Ser	Val	Ala	Ala	Ala	Gly	Gly	Leu	His	Glu	Phe	Ala	Asp	Ser	Gln	Phe
			5						10					15	
Gly	His	Cys	Phe	Ser	Trp	Gly	Glu	Asn	Arg	Glu	Glu	Ala	Ile	Ser	Asn
			20					25					30		
Met	Val	Val	Ala	Leu	Lys	Glu	Leu	Ser	Ile	Arg	Gly	Asp	Phe	Arg	Thr
		35					40					45			
Thr	Val	Glu	Tyr	Leu	Ile	Lys	Leu	Leu	Glu	Thr	Glu	Ser	Phe	Gln	Gln
	50					55					60				
Asn	Arg	Ile	Asp	Thr	Gly	Trp	Leu	Asp	Arg	Leu	Ile	Ala	Glu	Lys	Val
65					70					75					80
Gln	Ala	Glu	Arg	Pro	Asp	Thr	Met	Leu	Gly	Val	Val	Cys	Gly	Ala	Leu
				85					90					95	
His	Val	Ala	Asp	Val	Ser	Phe	Arg	Asn	Ser	Val	Ser	Asn	Phe	Leu	His
			100					105					110		
Ser	Leu	Glu	Arg	Gly	Gln	Val	Leu	Pro	Ala						
		115					120								

(2) INFORMATION FOR SEQ ID NO:29:

(i) Sequence characteristics:

(A) Length: 90 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:29:

Met	Val	Val	Ala	Leu ₅	Lys	Glu	Leu	Ser	Ile ₁₀	Arg	Gly	Asp	Phe	Arg ₁₅	Thr
Thr	Val	Glu	Tyr ₂₀	Leu	Ile	Lys	Leu	Leu ₂₅	Glu	Thr	Glu	Ser	Phe ₃₀	Gln	Gln
Asn	Arg	Ile ₃₅	Asp	Thr	Gly	Trp	Leu ₄₀	Asp	Arg	Leu	Ile	Ala ₄₅	Glu	Lys	Val

69

Gln Ala Glu Arg Pro Asp Thr Met Leu Gly Val Val Cys Gly Ala Leu
 50 55 60
 His Val Ala Asp Val Ser Phe Arg Asn Ser Val Ser Asn Phe Leu His
 65 70 75 80
 Ser Leu Glu Arg Gly Gln Val Leu Pro Ala
 85 90

(2) INFORMATION FOR SEQ ID NO:30:

(i) Sequence characteristics:

(A) Length: 190 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:30:

His Thr Leu Leu Asn Thr Val Asp Val Glu Leu Ile Tyr Glu Gly Arg
 5 10 15
 Lys Tyr Val Leu Lys Val Thr Arg Gln Ser Pro Asn Ser Tyr Val Val
 20 25 30
 Ile Met Asn Ser Ser Cys Val Glu Val Asp Val His Arg Leu Ser Asp
 35 40 45
 Gly Gly Leu Leu Leu Ser Tyr Asp Gly Ser Ser Tyr Thr Thr Tyr Met
 50 55 60
 Lys Glu Glu Val Asp Arg Tyr Arg Ile Thr Ile Gly Asn Lys Thr Cys
 65 70 75 80
 Val Phe Glu Lys Glu Asn Asp Pro Ser Ile Leu Arg Ser Pro Ser Ala
 85 90 95
 Gly Lys Leu Ile Gln Tyr Val Val Glu Asp Gly Gly His Val Phe Ala
 100 105 110
 Gly Gln Cys Phe Ala Glu Ile Glu Val Met Lys Met Val Met Thr Leu
 115 120 125
 Thr Ala Gly Glu Ser Gly Cys Ile His Tyr Val Lys Arg Pro Gly Ala
 130 135 140
 Val Leu Asp Pro Gly Cys Val Ile Ala Lys Leu Gln Leu Asp Asp Pro
 145 150 155 160
 Ser Arg Val Gln Gln Ala Glu Leu His Thr Gly Thr Leu Pro Gln Ile
 165 170 175
 Gln Ser Thr Ala Leu Arg Gly Glu Lys Leu His Arg Ile Phe
 180 185 190

(i) Sequence characteristics:

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:31:

(2) INFORMATION FOR SEQ ID NO:32:

(i) Sequence characteristics:

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:32:

[illegible]

71

(2) INFORMATION FOR SEQ ID NO:33:

(i) Sequence characteristics:

(A) Length: 73 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:33:

Val Asp Glu Lys Thr Gly Leu Val Ser Val Asp Asp Asp Ile Tyr Gln
 5 10 15
 Lys Gly Cys Cys Thr Ser Pro Glu Asp Gly Leu Gln Lys Ala Lys Arg
 20 25 30
 Ile Gly Phe Pro Val Met Ile Lys Ala Ser Glu Gly Gly Gly Lys
 35 40 45
 Gly Ile Arg Gln Val Glu Arg Glu Glu Asp Phe Ile Ala Leu Tyr His
 50 55 60
 Gln Ala Ala Asn Glu Ile Pro Gly Ser
 65 70

(2) INFORMATION FOR SEQ ID NO:34:

(i) Sequence characteristics:

(A) Length: 157 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:34:

Pro Ile Phe Ile Met Lys Leu Ala Gly Arg Ala Arg His Leu Glu Val
 5 10 15
 Gln Leu Leu Ala Asp Gln Tyr Gly Thr Asn Ile Ser Leu Phe Gly Arg
 20 25 30
 Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile Ile Glu Glu Ala Pro
 35 40 45
 Val Thr Ile Ala Lys Ala Glu Thr Phe His Glu Met Glu Lys Ala Ala
 50 55 60
 Val Arg Leu Gly Lys Leu Val Gly Tyr Val Ser Ala Gly Thr Val Glu
 65 70 75 80
 Tyr Leu Tyr Ser His Asp Asp Gly Lys Phe Tyr Phe Leu Glu Leu Asn
 85 90 95

72

Pro Arg Leu Gln Val Glu His Pro Thr Thr Glu Met Val Ser Gly Val
 100 105 110

Asn Leu Pro Ala Ala Gln Leu Gln Ile Ala Met Gly Ile Pro Met His
 115 120 125

Arg Ile Ser Asp Ile Arg Thr Leu Tyr Gly Met Asn Pro His Ser Ala
 130 135 140

Ser Glu Ile Asp Phe Glu Phe Lys Thr Gln Asp Ala Thr
 145 150 155

(2) INFORMATION FOR SEQ ID NO:35:

(i) Sequence characteristics:

(A) Length: 27 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:35:

Lys Lys Gln Arg Arg Pro Ile Pro Lys Gly His Cys Thr Ala Cys Arg
 5 10 15

Ile Thr Ser Glu Asp Pro Asn Asp Gly Phe Lys
 20 25

(2) INFORMATION FOR SEQ ID NO:36:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:36:

Pro Ser Gly Gly Thr Leu His Glu Leu Asn Phe Arg Ser Ser Ser Asn
 5 10 15

Val Trp Gly Tyr Phe
 20

(2) INFORMATION FOR SEQ ID NO:37:

(i) Sequence characteristics:

(A) Length: 122 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:37:

Ser	Val	Gly	Asn	Asn	Gly	Asn	Ile	His	Ser	Phe	Ser	Asp	Ser	Gln	Phe
			5						10						
Gly	His	Ile	Phe	Ala	Phe	Gly	Glu	Asn	Arg	Gln	Ala	Ser	Arg	Lys	His
			20					25					30		
Met	Val	Val	Ala	Leu	Lys	Glu	Leu	Ser	Ile	Arg	Gly	Asp	Phe	Arg	Thr
		35					40					45			
Thr	Val	Glu	Tyr	Leu	Ile	Lys	Leu	Leu	Glu	Thr	Glu	Asp	Phe	Glu	Asp
		50				55					60				
Asn	Thr	Ile	Thr	Thr	Gly	Trp	Leu	Asp	Asp	Leu	Ile	Thr	His	Lys	Met
65					70					75					80
Thr	Ala	Glu	Lys	Pro	Asp	Pro	Thr	Leu	Ala	Val	Ile	Cys	Gly	Ala	Ala
				85					90					95	
Thr	Lys	Ala	Phe	Leu	Ala	Ser	Glu	Glu	Ala	Arg	His	Lys	Tyr	Ile	Glu
			100					105					110		
Ser	Leu	Gln	Lys	Gly	Gln	Val	Leu	Ser	Lys						
		115					120								

(2) INFORMATION FOR SEQ ID NO:38:

(i) Sequence characteristics:

(A) Length: 190 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:38:

Asp	Leu	Leu	Gln	Thr	Met	Phe	Pro	Val	Asp	Phe	Ile	His	Glu	Gly	Lys
			5						10					15	
Arg	Tyr	Lys	Phe	Thr	Val	Ala	Lys	Ser	Gly	Asn	Asp	Arg	Tyr	Thr	Leu
			20					25					30		
Phe	Ile	Asn	Gly	Ser	Lys	Cys	Asp	Ile	Ile	Leu	Arg	Gln	Leu	Ser	Asp
		35					40					45			

74

Gly Gly Leu Leu Ile Ala Ile Gly Gly Lys Ser His Thr Ile Tyr Trp
 50 55 60
 Lys Glu Glu Val Ala Ala Thr Arg Leu Ser Val Asp Ser Met Thr Thr
 65 70 75 80
 Leu Leu Glu Val Glu Asn Asp Pro Thr Gln Leu Arg Thr Pro Ser Pro
 85 90 95
 Gly Lys Leu Val Lys Phe Leu Val Glu Asn Gly Glu His Ile Ile Lys
 100 105 110
 Gly Gln Pro Tyr Ala Glu Ile Glu Val Met Lys Met Gln Met Pro Leu
 115 120 125
 Val Ser Gln Glu Asn Gly Ile Val Gln Leu Leu Lys Gln Pro Gly Ser
 130 135 140
 Thr Ile Val Ala Gly Asp Ile Met Ala Ile Met Thr Leu Asp Asp Pro
 145 150 155 160
 Ser Lys Val Lys His Ala Leu Pro Phe Glu Gly Met Leu Pro Asp Phe
 165 170 175
 Gly Ser Pro Val Ile Glu Gly Thr Lys Pro Ala Tyr Lys Phe
 180 185 190

(2) INFORMATION FOR SEQ ID NO:39:

(i) Sequence characteristics:

(A) Length: 37 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:39:

Met Arg Phe Asn Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Leu
 5 10 15
 Arg Ile Leu Arg Thr Cys Glu Glu Leu Gly Ile Gly Thr Ile Ala Val
 20 25 30
 His Ser Thr Val Asp
 35

75

(2) INFORMATION FOR SEQ ID NO:40:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:40:

Arg Asn Ala Leu His Val Gln Leu Ala Asp Glu Ala Val Cys Ile Gly
 5 10 15
 Glu Ala Ala Ser Ser
 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) Sequence characteristics:

(A) Length: 38 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:41:

Lys Ser Tyr Leu Asn Ile Pro Asn Ile Ile Ala Ala Ala Leu Thr Arg
 5 10 15
 Asn Ala Ser Ala Ile His Pro Gly Tyr Gly Phe Leu Ala Glu Asn Ala
 20 25 30
 Arg Phe Ala Glu Ile Cys
 35

(2) INFORMATION FOR SEQ ID NO:42:

(i) Sequence characteristics:

(A) Length: 41 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:42:

Ala Asp His His Leu Thr Phe Ile Gly Pro Ser Pro Asp Ser Ile Arg
 5 10 15

76

Ala Met Gly Asp Lys Ser Thr Ala Lys Glu Thr Met Gln Arg Val Gly
 20 25 30
 Val Pro Thr Ile Pro Gly Ser Asp Gly
 35 40

(2) INFORMATION FOR SEQ ID NO:43:

(i) Sequence characteristics:

(A) Length: 143 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:43:

Leu Leu Thr Asp Val Asp Ser Ala Ala Lys Val Ala Ala Glu Ile Gly
 5 10 15
 Tyr Pro Val Met Ile Lys Ala Thr Ala Gly Gly Gly Gly Arg Gly Met
 20 25 30
 Arg Leu Val Arg Glu Pro Ala Asp Leu Glu Lys Leu Phe Leu Ala Ala
 35 40 45
 Gln Gly Glu Ala Glu Ala Ala Phe Gly Asn Pro Gly Leu Tyr Leu Glu
 50 55 60
 Lys Phe Ile Asp Arg Pro Arg His Val Glu Phe Gln Ile Leu Ala Asp
 65 70 75 80
 Ala Tyr Gly Asn Val Val His Leu Gly Glu Arg Asp Cys Ser Ile Gln
 85 90 95
 Arg Arg His Gln Lys Leu Leu Glu Glu Ala Pro Ser Pro Ala Leu Ser
 100 105 110
 Ala Asp Leu Arg Gln Lys Met Gly Asp Ala Ala Val Lys Val Ala Gln
 115 120 125
 Ala Ile Gly Tyr Ile Gly Ala Gly Thr Val Glu Phe Leu Val Asp
 130 135 140

(i) Sequence characteristics:

(A) Length: 50 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:44:

[illegible]

(i) Sequence characteristics:

(A) Length: 19 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:45:

Leu Arg Gly His Ala Ile Glu Cys Arg Ile Asn Ala Glu Asp Pro Glu
5 10 15
Tyr Asn Phe

78

(2) INFORMATION FOR SEQ ID NO:46:

(i) Sequence characteristics:

(A) Length: 9 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:46:

Arg Pro Asn Pro Gly Arg Ile Thr Gly
 5

(2) INFORMATION FOR SEQ ID NO:47:

(i) Sequence characteristics:

(A) Length: 7 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:47:

Pro Gly Val Arg Val Asp Ser
 5

(2) INFORMATION FOR SEQ ID NO:48:

(i) Sequence characteristics:

(A) Length: 44 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:48:

His Val Tyr Thr Asp Tyr Glu Ile Pro Pro Tyr Tyr Asp Ser Leu Ile
 5 10 15

Gly Lys Leu Ile Val Trp Gly Ala Thr Arg Glu Glu Ala Ile Ala Arg
 20 25 30

Met Gln Arg Ala Leu Arg Glu Cys Ala Ile Thr Gly
 35 40

(i) Sequence characteristics:

(A) Length: 38 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:49:

[illegible]

(i) Sequence characteristics:

(A) Length: 37 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:50:

Met Lys Phe Asp Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Leu
5 10 15
Arg Ile Leu Arg Ala Cys Glu Glu Met Gly Ile Ala Thr Ile Ala Val
20 25 30
His Ser Thr Val Asp
35

80

(2) INFORMATION FOR SEQ ID NO:51:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:51:

Arg Asn Ala Leu His Val Gln Leu Ala Asp Glu Ala Val Cys Ile Gly
 5 10 15

Glu Pro Ala Ser Ala
 20

(2) INFORMATION FOR SEQ ID NO:52:

(i) Sequence characteristics:

(A) Length: 38 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:52:

Lys Ser Tyr Leu Asn Ile Pro Asn Ile Ile Ala Ala Ala Leu Thr Arg
 5 10 15

Asn Ala Ser Ala Ile His Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ala
 20 25 30

Lys Phe Ala Glu Ile Cys
 35

(i) Sequence characteristics:

(A) Length: 42 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:53:

Ala	Asp	His	His	Ile	Ala	Phe	Ile	Gly	Pro	Thr	Pro	Glu	Ala	Ile	Arg
				5					10					15	
Leu	Met	Gly	Asp	Lys	Ser	Thr	Ala	Lys	Glu	Thr	Met	Gln	Lys	Ala	Gly
			20					25					30		
Val	Pro	Thr	Val	Pro	Gly	Ser	Glu	Gly	Leu						
		35					40								

(i) Sequence characteristics:

(A) Length: 142 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:54:

Val	Glu	Thr	Glu	Gln 5	Glu	Gly	Leu	Glu	Leu 10	Ala	Lys	Asp	Ile	Gly 15	Tyr
Pro	Val	Met	Ile 20	Lys	Ala	Thr	Ala	Gly 25	Gly	Gly	Gly	Arg	Gly 30	Met	Arg
Leu	Val	Arg	Ser 35	Pro	Asp	Glu	Phe 40	Val	Lys	Leu	Phe	Leu 45	Ala	Ala	Gln
Gly	Glu 50	Ala	Gly	Ala	Ala	Phe 55	Gly	Asn	Ala	Gly	Val 60	Tyr	Ile	Glu	Lys
Phe 65	Ile	Glu	Arg	Pro	Arg 70	His	Ile	Glu	Phe	Gln 75	Ile	Leu	Ala	Asp	Asn 80
Tyr	Gly	Asn	Val	Ile 85	His	Leu	Gly	Glu	Arg 90	Asp	Cys	Ser	Ile	Gln 95	Arg
Arg	Asn	Gln	Lys 100	Leu	Leu	Glu	Glu	Ala 105	Pro	Ser	Pro	Ala	Leu 110	Asp	Ser

82

Asp Leu Arg Glu Lys Met Gly Gln Ala Ala Val Lys Ala Ala Gln Phe
 115 120 125
 Ile Asn Tyr Ala Gly Ala Gly Thr Ile Glu Phe Leu Leu Asp
 130 135 140

(2) INFORMATION FOR SEQ ID NO:55:

(i) Sequence characteristics:

(A) Length: 50 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:55:

Arg Ser Gly Gln Phe Tyr Phe Met Glu Met Asn Thr Arg Ile Gln Val
 5 10 15
 Glu His Pro Val Thr Glu Met Val Thr Gly Val Asp Leu Leu Val Glu
 20 25 30
 Gln Ile Arg Ile Ala Gln Gly Glu Arg Leu Arg Leu Thr Gln Asp Gln
 35 40 45
 Val Val
 50

(2) INFORMATION FOR SEQ ID NO:56:

(i) Sequence characteristics:

(A) Length: 19 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:56:

Leu Arg Gly His Ala Ile Glu Cys Arg Ile Asn Ala Glu Asp Pro Asp
 5 10 15
 His Asp Phe

(2) INFORMATION FOR SEQ ID NO:57:

(i) Sequence characteristics:

(A) Length: 9 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:57:

Arg Pro Ala Pro Gly Arg Ile Ser Gly
5

(2) INFORMATION FOR SEQ ID NO:58:

(i) Sequence characteristics:

(A) Length: 6 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:58:

Tyr Leu Pro Pro Gly Gly
5

(2) INFORMATION FOR SEQ ID NO:59:

(i) Sequence characteristics:

(A) Length: 7 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:59:

Pro Gly Val Arg Ile Asp Ser
5

(i) **Sequence characteristics:**

(A) Length: 44 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:60:

His	Val	Tyr	Thr	Asp 5	Tyr	Gln	Ile	Pro	Pro 10	Tyr	Tyr	Asp	Ser	Leu 15	Ile
Gly	Lys	Leu	Ile 20	Val	Trp	Gly	Pro	Asp 25	Arg	Ala	Thr	Ala	Ile 30	Asn	Arg
Met	Lys	Arg 35	Ala	Leu	Arg	Glu	Cys 40	Ala	Ile	Thr	Gly				

(i) Sequence characteristics:

(A) Length: 154 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:61:

Leu	Pro	Thr	Thr	Ile 5	Gly	Phe	His	Gln	Arg 10	Ile	Met	Glu	Asn	Pro 15	Gln
Phe	Leu	Gln	Gly 20	Asn	Val	Ser	Thr	Ser 25	Phe	Val	Gln	Glu	Met 30	Asn	Lys
Pro	Leu	Asp 35	Phe	Asn	Glu	Ile	Arg 40	Gln	Leu	Leu	Thr	Thr 45	Ile	Ala	Gln
Thr	Asp 50	Ile	Ala	Glu	Val	Thr 55	Leu	Lys	Ser	Asp	Asp 60	Phe	Glu	Leu	Thr
Val 65	Arg	Lys	Ala	Val	Gly 50	Val	Asn	Asn	Ser	Val 75	Val	Pro	Val	Val	Thr 80
Ala	Pro	Leu	Ser	Gly 85	Val	Val	Gly	Ser	Gly 90	Leu	Pro	Ser	Ala	Ile 95	Pro
Ile	Val	Ala	His 100	Ala	Ala	Pro	Ser	Pro 105	Ser	Pro	Glu	Pro	Gly 110	Thr	Ser
Arg	Ala	Ala 115	Asp	His	Ala	Val	Thr 120	Ser	Ser	Gly	Ser	Gln 125	Pro	Gly	Ala

85

Lys Ile Ile Asp Gln Lys Leu Ala Glu Val Ala Ser Pro Met Val Gly
 130 135 140
 Thr Phe Tyr Arg Ala Pro Ala Pro Gly Glu
 145 150

(2) INFORMATION FOR SEQ ID NO:62:

(i) Sequence characteristics:

(A) Length: 24 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:62:

Ala Val Phe Val Glu Val Gly Asp Arg Ile Arg Gln Gly Gln Thr Val
 5 10 15
 Cys Ile Ile Glu Ala Met Lys Met
 20

(2) INFORMATION FOR SEQ ID NO:63:

(i) Sequence characteristics:

(A) Length: 36 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:63:

Met Leu Asp Lys Ile Val Ile Ala Asn Arg Gly Glu Ile Ala Leu Arg
 5 10 15
 Ile Leu Arg Ala Cys Lys Glu Leu Gly Ile Lys Thr Val Ala Val His
 20 25 30
 Ser Ser Ala Asp
 35

(i) Sequence characteristics:

(A) Length: 21 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:64:

Arg Asp Leu Lys His Val Leu Leu Ala Asp Glu Thr Val Cys Ile Gly
5 10 15

Pro Ala Pro Ser Val
20

(i) Sequence characteristics:

(A) Length: 38 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:65:

Lys Ser Tyr Leu Asn Ile Pro Ala Ile Ile Ser Ala Ala Glu Ile Thr
5 10 15

Gly Ala Val Ala Ile His Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ala
20 25 30

Asn Phe Ala Glu Gln Val
35

(i) Sequence characteristics:

(A) Length: 43 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:66:

Glu Arg Ser Gly Phe Ile Phe Ile Gly Pro Lys Ala Glu Thr Ile Arg
5 10 15

87

Leu Met Gly Asp Lys Val Ser Ala Ile Ala Ala Met Lys Lys Ala Gly
 20 25 30
 Val Pro Cys Val Pro Gly Ser Asp Gly Pro Leu
 35 40

(2) INFORMATION FOR SEQ ID NO:67:

(i) Sequence characteristics:

(A) Length: 141 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:67:

Gly Asp Asp Met Asp Lys Asn Arg Ala Ile Ala Lys Arg Ile Gly Tyr
 5 10 15
 Pro Val Ile Ile Lys Ala Ser Gly Gly Gly Gly Arg Gly Met Arg
 20 25 30
 Val Val Arg Gly Asp Ala Glu Leu Ala Gln Ser Ile Ser Met Thr Arg
 35 40 45
 Ala Glu Ala Lys Ala Ala Phe Ser Asn Asp Met Val Tyr Met Glu Lys
 50 55 60
 Tyr Leu Glu Asn Pro Arg His Val Glu Ile Gln Val Leu Ala Asp Gly
 65 70 75 80
 Gln Gly Asn Ala Ile Tyr Leu Ala Glu Arg Asp Cys Ser Met Gln Arg
 85 90 95
 Arg His Gln Lys Val Val Glu Glu Ala Pro Ala Pro Gly Ile Thr Pro
 100 105 110
 Glu Leu Arg Arg Tyr Ile Gly Glu Arg Cys Ala Lys Ala Cys Val Asp
 115 120 125
 Ile Gly Tyr Arg Gly Ala Gly Thr Phe Glu Phe Leu Phe
 130 135 140

(i) Sequence characteristics:

(A) Length: 50 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:68:

[illegible]

(i) Sequence characteristics:

(A) Length: 25 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:69:

Val Arg Gly His Ala Val Glu Cys Arg Ile Asn Ala Glu Asp Pro Asn
5 10 15

Leu Pro Ser Pro Gly Lys Ile Thr Arg
20 25

89

(2) INFORMATION FOR SEQ ID NO:70:

(i) Sequence characteristics:

(A) Length: 6 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:70:

Phe His Ala Pro Gly Gly
 5

(2) INFORMATION FOR SEQ ID NO:71:

(i) Sequence characteristics:

(A) Length: 7 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:71:

Phe Gly Val Arg Trp Glu Ser
 5

(2) INFORMATION FOR SEQ ID NO:72:

(i) Sequence characteristics:

(A) Length: 44 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:72:

His Ile Tyr Ala Gly Tyr Thr Val Pro Pro Tyr Tyr Asp Ser Met Ile
 5 10 15

Gly Lys Leu Ile Cys Tyr Gly Glu Asn Arg Asp Val Ala Ile Ala Arg
 20 25 30

Met Lys Asn Ala Leu Gln Glu Leu Ile Ile Asp Gly
 35 40

90

(2) INFORMATION FOR SEQ ID NO:73:**(i) Sequence characteristics:**

(A) Length: 135 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide**(xi) Sequence Description: SEQ ID NO:73:**

```

Ile Lys Thr Asn Val Asp Leu Gln Ile Arg Ile Met Asn Asp Glu Asn
      5              10              15
Phe Gln His Gly Gly Thr Asn Ile His Tyr Leu Glu Lys Lys Leu Gly
      20              25              30
Leu Gln Glu Lys Met Asp Ile Arg Lys Ile Lys Lys Leu Ile Glu Leu
      35              40              45
Val Glu Glu Ser Gly Ile Ser Glu Leu Glu Ile Ser Glu Gly Glu Glu
      50              55              60
Ser Val Arg Ile Ser Arg Ala Ala Pro Ala Ala Ser Phe Pro Val Met
      65              70              75              80
Gln Gln Ala Tyr Ala Ala Pro Met Met Gln Gln Pro Ala Gln Ser Asn
      85              90              95
Ala Ala Ala Pro Ala Thr Val Pro Ser Met Glu Ala Pro Ala Ala Ala
      100             105             110
Glu Ile Ser Gly His Ile Val Arg Ser Pro Met Val Gly Thr Phe Tyr
      115             120             125
Arg Thr Pro Ser Pro Asp Ala
      130             135

```

(2) INFORMATION FOR SEQ ID NO:74:**(i) Sequence characteristics:**

(A) Length: 57 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide**(xi) Sequence Description: SEQ ID NO:74:**

```

Lys Ala Phe Ile Glu Val Gly Gln Lys Val Asn Val Gly Asp Thr Leu
      5              10              15
Cys Ile Val Glu Ala Met Lys Met Met Asn Gln Ile Glu Ala Asp Lys
      20              25              30

```

91

Ser Gly Thr Val Lys Ala Ile Leu Val Glu Ser Gly Gln Pro Val Glu
 35 40 45
 Phe Asp Glu Pro Leu Val Val Ile Glu
 50 55

(2) INFORMATION FOR SEQ ID NO:75:

(i) Sequence characteristics:

(A) Length: 72 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:75:

Met Leu Ser Ala Ala Leu Arg Thr Leu Lys His Val Leu Tyr Tyr Ser
 5 10 15
 Arg Gln Cys Leu Met Val Ser Arg Asn Leu Gly Ser Val Gly Tyr Asp
 20 25 30
 Pro Asn Glu Lys Thr Phe Asp Lys Ile Leu Val Ala Asn Arg Gly Glu
 35 40 45
 Ile Ala Cys Arg Val Ile Arg Thr Cys Lys Lys Met Gly Ile Lys Thr
 50 55 60
 Val Ala Ile His Ser Asp Val Asp
 65 70

(2) INFORMATION FOR SEQ ID NO:76:

(i) Sequence characteristics:

(A) Length: 21 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:76:

Ala Ser Ser Val His Val Lys Met Ala Asp Glu Ala Val Cys Val Gly
 5 10 15
 Pro Ala Pro Thr Ser
 20

92

(2) INFORMATION FOR SEQ ID NO:77:

(i) Sequence characteristics:

(A) Length: 38 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:77:

Lys Ser Tyr Leu Asn Met Asp Ala Ile Met Glu Ala Ile Lys Lys Thr
 5 10 15
 Arg Ala Gln Ala Val His Pro Gly Tyr Gly Phe Leu Ser Glu Asn Lys
 20 25 30
 Glu Phe Ala Arg Cys Leu
 35

(2) INFORMATION FOR SEQ ID NO:78:

(i) Sequence characteristics:

(A) Length: 41 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:78:

Ala Ala Glu Asp Val Val Phe Ile Gly Pro Asp Thr His Ala Ile Gln
 5 10 15
 Ala Met Gly Asp Lys Ile Glu Ser Lys Leu Leu Ala Lys Lys Ala Glu
 20 25 30
 Val Asn Thr Ile Pro Gly Phe Asp Gly
 35 40

(2) INFORMATION FOR SEQ ID NO:79:

(i) Sequence characteristics:

(A) Length: 144 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:79:

```

Val Lys Asp Ala Glu Glu Ala Val Arg Ile Ala Arg Glu Ile Gly Tyr
      5              10              15
Pro Val Met Ile Lys Ala Ser Ala Gly Gly Gly Gly Lys Gly Met Arg
      20              25              30
Ile Ala Trp Asp Asp Glu Glu Thr Arg Asp Gly Phe Arg Leu Ser Ser
      35              40              45
Gln Glu Ala Ala Ser Ser Phe Gly Asp Asp Arg Leu Leu Ile Glu Lys
      50              55              60
Phe Ile Asp Asn Pro Arg His Ile Glu Ile Gln Val Leu Gly Asp Lys
      65              70              75
His Gly Asn Ala Leu Trp Leu Asn Glu Arg Glu Cys Ser Ile Gln Arg
      85              90              95
Arg Asn Gln Lys Val Val Glu Glu Ala Pro Ser Ile Phe Leu Asp Ala
      100             105             110
Glu Thr Arg Arg Ala Met Gly Glu Gln Ala Val Ala Leu Ala Arg Ala
      115             120             125
Val Lys Tyr Ser Ser Ala Gly Thr Val Glu Phe Leu Val Asp Ser Lys
      130             135             140

```

(i) Sequence characteristics:

(A) Length: 47 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:80:

Lys	Asn	Phe	Tyr	Phe	Leu	Glu	Met	Asn	Thr	Arg	Leu	Gln	Val	Glu	His
				5					10					15	
Pro	Val	Thr	Glu	Cys	Ile	His	Trp	Pro	Gly	Pro	Ser	Pro	Gly	Lys	Thr
			20					25					30		
Val	Leu	Gln	Glu	His	Leu	Ser	Gly	Thr	Asn	Lys	Leu	Ile	Phe	Ala	
		35					40					45			

(i) Sequence characteristics:

(A) Length: 29 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:81:

Phe Asn Gly Trp Ala Val Glu Cys Arg Val Tyr Ala Glu Asp Pro Tyr
5 10 15
Lys Ser Phe Gly Leu Pro Ser Ile Gly Arg Leu Ser Gln
20 25

(i) **Sequence characteristics:**

(A) Length: 14 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:82:

Tyr Gln Glu Pro Leu His Leu Pro Gly Val Arg Val Asp Ser
5 10

(2) INFORMATION FOR SEQ ID NO:83:

(i) Sequence characteristics:

(A) Length: 44 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:83:

Gly Ile Gln Pro Gly Ser Asp Ile Ser Ile Tyr Tyr Asp Pro Met Ile
5 10 15
Ser Lys Leu Ile Thr Tyr Gly Ser Asp Arg Thr Glu Ala Leu Lys Arg
20 25 30
Met Ala Asp Ala Leu Asp Asn Tyr Val Ile Arg Gly
35 40

(2) INFORMATION FOR SEQ ID NO:84:

(i) Sequence characteristics:

(A) Length: 251 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:84:

Val	Thr	His	Asn	Ile	Ala	Leu	Leu	Arg	Glu	Val	Ile	Ile	Asn	Ser	Arg
			5						10					15	
Phe	Val	Lys	Gly	Asp	Ile	Ser	Thr	Lys	Phe	Leu	Ser	Asp	Val	Tyr	Pro
			20					25					30		
Asp	Gly	Phe	Lys	Gly	His	Met	Leu	Thr	Lys	Ser	Glu	Lys	Asn	Gln	Leu
		35					40					45			
Leu	Ala	Ile	Ala	Ser	Ser	Leu	Phe	Val	Ala	Phe	Gln	Leu	Arg	Ala	Gln
	50					55					60				
His	Phe	Gln	Glu	Asn	Ser	Arg	Met	Pro	Val	Ile	Lys	Pro	Asp	Ile	Ala
65					70					75					80
Asn	Trp	Glu	Leu	Ser	Val	Lys	Leu	His	Asp	Lys	Val	His	Thr	Val	Val
				85					90					95	
Ala	Ser	Asn	Asn	Gly	Ser	Val	Phe	Ser	Val	Glu	Val	Asp	Gly	Ser	Lys
			100					105					110		
Leu	Asn	Val	Thr	Ser	Thr	Trp	Asn	Leu	Ala	Ser	Pro	Leu	Leu	Ser	Val
		115					120					125			

96

Ser Val Asp Gly Thr Gln Arg Thr Val Gln Cys Leu Ser Arg Glu Ala
 130 135 140
 Gly Gly Asn Met Ser Ile Gln Phe Leu Gly Thr Val Tyr Lys Val Asn
 145 150 155 160
 Ile Leu Thr Arg Leu Ala Ala Glu Leu Asn Lys Phe Met Leu Glu Lys
 165 170 175
 Val Thr Glu Asp Thr Ser Ser Val Leu Arg Ser Pro Met Pro Gly Val
 180 185 190
 Val Val Ala Val Ser Val Lys Pro Gly Asp Ala Val Ala Glu Gly Gln
 195 200 205
 Glu Ile Cys Val Ile Glu Ala Met Lys Met Gln Asn Ser Met Thr Ala
 210 215 220
 Gly Lys Thr Gly Thr Val Lys Ser Val His Cys Gln Ala Gly Asp Thr
 225 230 235 240
 Val Gly Glu Gly Asp Leu Leu Val Glu Leu Glu
 245 250

(2) INFORMATION FOR SEQ ID NO:85:

(i) Sequence characteristics:

(A) Length: 90 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:85:

Met Pro Tyr Arg Glu Arg Phe Cys Ala Ile Arg Trp Cys Arg Asn Ser
 5 10 15
 Gly Arg Ser Ser Gln Gln Leu Leu Trp Thr Leu Lys Arg Ala Pro Val
 20 25 30
 Tyr Ser Gln Cys Leu Val Val Ser Arg Ser Leu Ser Ser Val Glu
 35 40 45
 Tyr Glu Pro Lys Glu Lys Thr Phe Asp Lys Ile Leu Ile Ala Asn Arg
 50 55 60
 Gly Glu Ile Ala Cys Arg Val Ile Lys Thr Cys Arg Lys Met Gly Ile
 65 70 75 80
 Arg Thr Val Ala Ile His Ser Asp Val Asp
 85 90

(i) Sequence characteristics:

(A) Length: 21 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:86:

Ala Ser Ser Val His Val Lys Met Ala Asp Glu Ala Val Cys Val Gly
5 10 15

Pro Ala Pro Thr Ser
20

(i) Sequence characteristics:

(A) Length: 38 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:87:

Lys Ser Tyr Leu Asn Met Asp Ala Ile Met Glu Ala Ile Lys Lys Thr
5 10 15
Gly Ala Gln Ala Val His Pro Gly Tyr Gly Phe Leu Ser Glu Asn Lys
20 25 30
Glu Phe Ala Lys Cys Leu
35

(i) Sequence characteristics:

(A) Length: 41 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:88:

Ala Ala Glu Asp Val Thr Phe Ile Gly Pro Asp Thr His Ala Ile Gln
5 10 15

98

Ala Met Gly Asp Lys Ile Glu Ser Lys Leu Leu Ala Lys Arg Ala Lys
 20 25 30
 Val Asn Thr Ile Pro Gly Phe Asp Gly
 35 40

(2) INFORMATION FOR SEQ ID NO:89:

(i) Sequence characteristics:

(A) Length: 144 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:89:

Leu Lys Asp Ala Asp Glu Ala Val Arg Ile Ala Arg Glu Ile Gly Tyr
 5 10 15
 Pro Val Met Ile Lys Ala Ser Ala Gly Gly Gly Gly Lys Gly Met Arg
 20 25 30
 Ile Pro Trp Asp Asp Glu Glu Thr Arg Asp Gly Phe Arg Phe Ser Ser
 35 40 45
 Gln Glu Ala Ala Ser Ser Phe Gly Asp Asp Arg Leu Leu Ile Glu Lys
 50 55 60
 Phe Ile Asp Asn Pro Arg His Ile Glu Ile Gln Val Leu Gly Asp Lys
 65 70 75 80
 His Gly Asn Ala Leu Trp Leu Asn Glu Arg Glu Cys Ser Ile Gln Arg
 85 90 95
 Arg Asn Gln Lys Val Val Glu Glu Ala Pro Ser Ile Phe Leu Asp Pro
 100 105 110
 Glu Thr Arg Arg Ala Met Gly Glu Gln Ala Val Ala Trp Pro Lys Ala
 115 120 125
 Val Lys Tyr Ser Ser Ala Gly Thr Val Glu Phe Leu Val Asp Ser Gln
 130 135 140

(i) Sequence characteristics:

(A) Length: 48 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:90:

Lys Asn Phe Tyr Phe Leu Glu Met Asn Thr Arg Leu Gln Val Glu His
5 10 15

Pro Val Thr Glu Cys Ile Thr Gly Leu Asp Leu Val Gln Glu Met Ile
20 25 30

Leu Val Ala Lys Gly Tyr Pro Leu Arg His Lys Gln Glu Asp Ile Pro
35 40 45

(1) Sequence characteristics:

(A) Length: 29 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:91:

Ile Ser Gly Trp Ala Val Glu Cys Arg Val Tyr Ala Glu Asp Pro Tyr
5 10 15

Lys Ser Phe Gly Leu Pro Ser Ile Gly Arg Leu Ser Gln
20 25

(i) Sequence characteristics:

(A) Length: 14 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:92:

Tyr Gln Glu Pro Ile His Leu Pro Gly Val Arg Val Asp Ser
5 10

100

(i) Sequence characteristics:

(A) Length: 44 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:93:

Gly Ile Gln Pro Gly Ser Asp Ile Ser Ile Tyr His Asp Pro Met Ile
5 10 15

Ser Lys Leu Val Thr Tyr Gly Ser Asp Arg Ala Glu Ala Leu Lys Arg
20 25 30

Met Glu Asp Ala Leu Asp Ser Tyr Val Ile Arg Gly
35 40

(i) Sequence characteristics:

(A) Length: 251 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:94:

Val	Thr	His	Asn	Ile	Pro	Leu	Leu	Arg	Glu	Val	Ile	Ile	Asn	Thr	Arg
			5						10					15	
Phe	Val	Lys	Gly	Asp	Ile	Ser	Thr	Lys	Phe	Leu	Ser	Asp	Val	Tyr	Pro
			20					25					30		
Asp	Gly	Phe	Lys	Gly	His	Met	Leu	Thr	Pro	Ser	Glu	Arg	Asp	Gln	Leu
		35					40					45			
Leu	Ala	Ile	Ala	Ser	Ser	Leu	Phe	Val	Ala	Ser	Gln	Leu	Arg	Ala	Gln
	50					55					60				
Arg	Phe	Gln	Glu	His	Ser	Arg	Val	Pro	Val	Ile	Arg	Pro	Asp	Val	Ala
65					70					75					80
Lys	Trp	Glu	Leu	Ser	Val	Lys	Leu	His	Asp	Glu	Asp	His	Thr	Val	Val
				85					90					95	
Ala	Ser	Asn	Asn	Gly	Pro	Thr	Phe	Asn	Val	Glu	Val	Asp	Gly	Ser	Lys
			100					105					110		
Leu	Asn	Val	Thr	Ser	Thr	Trp	Asn	Leu	Ala	Ser	Pro	Leu	Leu	Ser	Val
		115					120					125			

101

```

Asn Val Asp Gly Thr Gln Arg Thr Val Gln Cys Leu Ser Pro Asp Ala
 130          135          140

Gly Gly Asn Met Ser Ile Gln Phe Leu Gly Thr Val Tyr Lys Val His
 145          150          155          160

Ile Leu Thr Lys Leu Ala Ala Glu Leu Asn Lys Phe Met Leu Glu Lys
      165          170          175

Val Pro Lys Asp Thr Ser Ser Val Leu Arg Ser Pro Lys Pro Gly Val
      180          185          190

Val Val Ala Val Ser Val Lys Pro Gly Asp Met Val Ala Glu Gly Gln
      195          200          205

Glu Ile Cys Val Ile Glu Ala Met Lys Met Gln Asn Ser Met Thr Ala
 210          215          220

Gly Lys Met Gly Lys Val Lys Leu Val His Cys Lys Ala Gly Asp Thr
 225          230          235          240

Val Gly Glu Gly Asp Leu Leu Val Glu Leu Glu
      245          250

```

(2) INFORMATION FOR SEQ ID NO:95:

(i) Sequence characteristics:

```

(A) Length:      17 amino acids
(B) Type:        Amino acid
(C) Strandedness: Single
(D) Topology:    Linear

```

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:95:

```

Gln Arg Lys Phe Ala Gly Leu Arg Asp Asn Phe Asn Leu Leu Gly Glu
      5          10          15

```

Lys

102

(2) INFORMATION FOR SEQ ID NO:96:

(i) Sequence characteristics:

(A) Length: 34 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:96:

Asn Lys Ile Leu Val Ala Asn Arg Gly Glu Ile Pro Ile Arg Ile Phe
5 10 15
Arg Thr Ala His Glu Leu Ser Met Gln Thr Val Ala Ile Tyr Ser His
20 25 30
Glu Asp

(2) INFORMATION FOR SEQ ID NO:97:

(i) Sequence characteristics:

(A) Length: 24 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:97:

Arg Leu Ser Thr His Lys Gln Lys Ala Asp Glu Ala Tyr Val Ile Gly
5 10 15
Glu Val Gly Gln Tyr Thr Pro Val
20

(2) INFORMATION FOR SEQ ID NO:98:

(i) Sequence characteristics:

(A) Length: 38 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:98:

Gly Ala Tyr Leu Ala Ile Asp Glu Ile Ile Ser Ile Ala Gln Lys His
5 10 15
Gln Val Asp Phe Ile His Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ser
20 25 30
Glu Phe Ala Asp Lys Val
35

(2) INFORMATION FOR SEQ ID NO:99:

(i) Sequence characteristics:

(A) Length: 41 amino acids
(B) Type: Amino acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:99:

Val Lys Ala Gly Ile Thr Trp Ile Gly Pro Pro Ala Glu Val Ile Asp
5 10 15

Ser Val Gly Asp Lys Val Ser Ala Arg Asn Leu Ala Ala Lys Ala Asn
20 25 30

Val Pro Thr Val Pro Gly Thr Pro Gly
35 40

104

(2) INFORMATION FOR SEQ ID NO:100:

(i) Sequence characteristics:

(A) Length: 144 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:100:

```

Ile Glu Thr Val Glu Glu Ala Leu Asp Phe Val Asn Glu Tyr Gly Tyr
      5                      10                      15
Pro Val Ile Ile Lys Ala Ala Phe Gly Gly Gly Gly Arg Gly Met Arg
      20                      25                      30
Val Val Arg Glu Gly Asp Asp Val Ala Asp Ala Phe Gln Arg Ala Thr
      35                      40                      45
Ser Glu Ala Arg Thr Ala Phe Gly Asn Gly Thr Cys Phe Val Glu Arg
      50                      55                      60
Phe Leu Asp Lys Pro Lys His Ile Glu Val Gln Leu Leu Ala Asp Asn
      65                      70                      75                      80
His Gly Asn Val Val His Leu Phe Glu Arg Asp Cys Ser Val Gln Arg
      85                      90                      95
Arg His Gln Lys Val Val Glu Val Ala Pro Ala Lys Thr Leu Pro Arg
      100                     105                     110
Glu Val Arg Asp Ala Ile Leu Thr Asp Ala Val Lys Leu Ala Lys Glu
      115                     120                     125
Cys Gly Tyr Arg Asn Ala Gly Thr Ala Glu Phe Leu Val Asp Asn Gln
      130                     135                     140

```

105

(2) INFORMATION FOR SEQ ID NO:101:

(i) Sequence characteristics:

(A) Length: 51 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:101:

Asn Arg His Tyr Phe Ile Glu Ile Asn Pro Arg Ile Gln Val Glu His
 5 10 15
 Thr Ile Thr Glu Glu Ile Thr Gly Ile Asp Ile Val Ala Ala Gln Ile
 20 25 30
 Gln Ile Ala Ala Gly Ala Ser Leu Pro Gln Leu Gly Leu Phe Gln Asp
 35 40 45
 Lys Ile Thr
 50

(2) INFORMATION FOR SEQ ID NO:102:

(i) Sequence characteristics:

(A) Length: 20 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:102:

Thr Arg Gly Phe Ala Ile Gln Cys Arg Ile Thr Thr Glu Asp Pro Ala
 5 10 15
 Lys Asn Phe Gln
 20

106

(2) INFORMATION FOR SEQ ID NO:103:**(i) Sequence characteristics:**

(A) Length: 14 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide**(xi) Sequence Description: SEQ ID NO:103:**

Pro Asp Thr Gly Arg Ile Glu Val Tyr Arg Ser Ala Gly Gly
 5 10

(2) INFORMATION FOR SEQ ID NO:104:**(i) Sequence characteristics:**

(A) Length: 52 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide**(xi) Sequence Description: SEQ ID NO:104:**

Asn Gly Val Arg Leu Asp Gly Gly Asn Ala Tyr Ala Gly Thr Ile Ile
 5 10 15

25 Ser Pro His Tyr Asp Ser Met Leu Val Lys Cys Ser Cys Ser Gly Ser
 20 30

Thr Tyr Glu Ile Val Arg Arg Lys Met Ile Arg Ala Leu Ile Glu Phe
 35 40 45

Arg Ile Arg Gly
 50

107

(2) INFORMATION FOR SEQ ID NO:105:

(i) Sequence characteristics:

(A) Length: 257 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:105:

Val Lys Thr Asn Ile Pro Phe Leu Leu Thr Leu Leu Thr Asn Pro Val
 5 10 15
 Phe Ile Glu Gly Thr Tyr Trp Gly Thr Phe Ile Asp Asp Thr Pro Gln
 20 25 30
 Leu Phe Gln Met Val Ser Ser Gln Asn Arg Ala Gln Lys Leu Leu His
 35 40 45
 Tyr Leu Ala Asp Val Ala Asp Asn Gly Ser Ser Ile Lys Gly Gln Ile
 50 55 60
 Gly Leu Pro Lys Leu Lys Ser Asn Pro Ser Val Pro His Ser Tyr Asn
 65 70 75 80
 Met Tyr Pro Arg Val Tyr Glu Asp Phe Gln Lys Met Arg Glu Thr Tyr
 85 90 95
 Gly Asp Leu Ser Val Leu Pro Thr Arg Ser Phe Leu Ser Pro Leu Glu
 100 105 110
 Thr Asp Glu Glu Ile Glu Val Val Ile Glu Gln Gly Lys Thr Leu Ile
 115 120 125
 Ile Lys Leu Gln Ala Val Gly Asp Leu Asn Lys Lys Thr Gly Glu Arg
 130 135 140
 Glu Val Tyr Phe Asp Leu Asn Gly Glu Met Arg Lys Ile Arg Val Ala
 145 150 155 160
 Asp Arg Ser Gln Lys Val Glu Thr Val Thr Lys Ser Lys Ala Asp Met
 165 170 175
 His Asp Pro Leu His Ile Gly Ala Pro Met Ala Gly Val Ile Val Glu
 180 185 190
 Val Lys Val His Lys Gly Ser Leu Ile Lys Lys Gly Gln Pro Val Ala
 195 200 205
 Val Leu Ser Ala Met Lys Met Glu Met Ile Ile Ser Ser Pro Ser Asp
 210 215 220
 Gly Gln Val Lys Glu Val Phe Val Ser Asp Gly Glu Asn Val Asp Ser
 225 230 235 240
 Ser Asp Leu Leu Val Leu Leu Glu Asp Gln Val Pro Val Glu Thr Lys
 245 250 255

Ala

108

(2) INFORMATION FOR SEQ ID NO:106:

(i) Sequence characteristics:

(A) Length: 165 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:106:

Val Leu Thr Val Ala Leu Phe Pro Gln Pro Gly Leu Lys Phe Leu Glu
 5 10 15
 Asn Arg His Asn Pro Ala Ala Phe Glu Pro Val Pro Gln Ala Glu Ala
 20 25 30
 Ala Gln Pro Val Ala Lys Ala Glu Lys Pro Ala Ala Ser Gly Val Tyr
 35 40 45
 Thr Val Glu Val Glu Gly Lys Ala Phe Val Val Lys Val Ser Asp Gly
 50 55 60
 Gly Asp Val Ser Gln Leu Thr Ala Ala Ala Pro Ala Pro Ala Pro Ala
 65 70 75 80
 Pro Ala Pro Ala Ser Ala Pro Ala Ala Ala Ala Pro Ala Gly Ala Gly
 85 90 95
 Thr Pro Val Thr Ala Pro Leu Ala Gly Thr Ile Trp Lys Val Leu Ala
 100 105 110
 Ser Glu Gly Gln Thr Val Ala Ala Gly Glu Val Leu Leu Ile Leu Glu
 115 120 125
 Ala Met Lys Met Glu Thr Glu Ile Arg Ala Ala Gln Ala Gly Thr Val
 130 135 140
 Arg Gly Ile Ala Val Lys Ala Gly Asp Ala Val Ala Val Gly Asp Thr
 145 150 155 160
 Leu Met Thr Leu Ala
 165

109

(2) INFORMATION FOR SEQ ID NO:107:

(i) Sequence characteristics:

(A) Length: 123 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(xi) Sequence Description: SEQ ID NO:107:

Met Lys Leu Lys Val Thr Val Asn Gly Thr Ala Tyr Asp Val Asp Val
 5 10 15
 Asp Val Asp Lys Ser His Glu Asn Pro Met Gly Thr Ile Leu Phe Gly
 20 25 30
 Gly Gly Thr Gly Gly Ala Pro Ala Pro Arg Ala Ala Gly Gly Ala Gly
 35 40 45
 Ala Gly Lys Ala Gly Glu Gly Glu Ile Pro Ala Pro Leu Ala Gly Thr
 50 55 60
 Val Ser Lys Ile Leu Val Lys Glu Gly Asp Thr Val Lys Ala Gly Gln
 65 70 75 80
 Thr Val Leu Val Leu Glu Ala Met Lys Met Glu Thr Glu Ile Asn Ala
 85 90 95
 Pro Thr Asp Gly Lys Val Glu Lys Val Leu Val Lys Glu Arg Asp Ala
 100 105 110
 Val Gln Gly Gly Gln Gly Leu Ile Lys Ile Gly
 115 120

(2) INFORMATION FOR SEQ ID NO:108:

(i) Sequence characteristics:

(A) Length: 1473 base pairs
(B) Type: Nucleic acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Oligonucleotide

(xi) Sequence Description: SEQ ID NO:108:

GTGATGATCA AGGCATCATG GGGTGGGGGT GGTAAGGAA TAAGGAAGGT ACATAATGAT 60
GATGAGGTCA GAGCATTGTT TAAGCAAGTG CAAGGAGAAG TCCCCGGATC GCCTATATTT 120
ATTATGAAGG TGGCATCTCA GAGTCGACAT CTAGAGGTTT AATTGCTCTG TGACAAGCAT 180
GGCAACGTGG CAGCACTGCA CAGTCGAGAC TGTAGTGTTT AAAGAAGGCA TCAAAGATC 240
ATTGAGGAGG GACCAATTAC AGTTGCTCCT CCAGAAACAA TTAAAGAGCT TGAGCAGGCG 300
GCAAGGCGAC TAGCTAAATG TGTGCAATAT CAGGGTGCTG CTACAGTGA ATATCTGTAC 360
AGCATGGAAG CAGGCGAATA CTATTCCTG GAGCTTAATC CAAGGTTGCA GGTAGAACAC 420
CCTGTGACCG AATGGATTGC TGAATAAAC TTACCYGCAT CTCAAGTTGT AGTAGGAATG 480
GGCATACCAC TCTACAACAT TCCAGAGATC AGACGCTTTT ATGGAATAGA ACATGGAGGT 540
GGCTATCAYG CTTGGAAGGA AATATCAGCT GTTGCAACTA AATTGATYT GGACAAAGCA 600
CAGTCTGTAA AGCCAAARGG TCATTGTGTA GCAGTTAGAG TTACTAGCGA GGATCCAGAT 660
GATGGGTTTA AGCCTACMAG TGGAAGAGTR GAAGAGCTGA ACTTTAAAAG TAAACCCAAT 720
GTTTGGGCCT ATTTCTCYGT TARGTCCGGA GGTGCAATTC AYGAGTTCTC TGATTCCCAG 780
TTTGGTTCATG TTTTGCTTY TGGGGAATCT AGGTCWTTGG CAATAGCCAA TATGGTACTT 840
GGGTTAAAAG AGATCCAAAT TCGTGGAGAG ATACGCACTA ATGTTGACTA CACTGTGGAT 900
CTCTTGAATG CTGCAGAGTA CCGAGAAAAT AWGATTCACA CTGGTTGGCT AGACAGCAGA 960

111

ATAGCWATGC GYGTTAGAGC AGAGAGGCCCC CCATGGTACC TTTCAGTTGT TGGTGGAGCT 1020
 CTATATGAAG CATCAAGCAG GAGCTCGAGT GTTGTAAACCG ATTATGTTGG TTATCTCAGT 1080
 AAAGGTCAAA TACCACCAAA GCACATCTCT CTTGTCAAYT TGA CTGTAAC ACTGAATATA 1140
 GATGGGAGCA AATATACGAT TGAGACAGTA CGAGGTGGAC CCCGTAGCTA CAAATTAAGA 1200
 ATTAATGAAT CAGAGGTTGA RGCAGAGATA CATTTCCTGC GAGATGGCGG ACYCTTAATG 1260
 CAGTYGGATG GAAACAGTCA TGTAATTTAC GCCGAGACAG AAGCTKCTGG CACGCGCCTT 1320
 CTAATCAATG GGAGAACATG CTTATTACAG AAAGAGCAYG ATCCTTCCAG GTTGTGGCT 1380
 GATACACCRT GCAARCTTCT TCGGTTTTTG GTCGCGGATR GTTCTCATGT GGTGCTGAT 1440
 ACGCCATATG CYGAGGTGGA GGCCATGAAA ATG 1473

(2) INFORMATION FOR SEQ ID NO:109:

(i) Sequence characteristics:

(A) Length: 491 amino acids
 (B) Type: Amino acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Peptide

(ix) Features

(A) NAME/KEY: Xaa
 (B) LOCATION: 248, 267, 311, 412, 418, 422, 436, and 474
 (C) IDENTIFICATION METHOD: Xaa = any amino acid

(xi) Sequence Description: SEQ ID NO:109:

Val Met Ile Lys Ala Ser Trp Gly Gly Gly Lys Gly Ile Arg Lys
 5 10 15
 Val His Asn Asp Asp Glu Val Arg Ala Leu Phe Lys Gln Val Gln Gly
 20 25 30
 Glu Val Pro Gly Ser Pro Ile Phe Ile Met Lys Val Ala Ser Gln Ser
 35 40 45
 Arg His Leu Glu Val Gln Leu Leu Cys Asp Lys His Gly Asn Val Ala
 50 55 60
 Ala Leu His Ser Arg Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile
 65 70 75 80

112

Ile Glu Glu Gly Pro Ile Thr Val Ala Pro Pro Glu Thr Ile Lys Glu
 85 90 95
 Leu Glu Gln Ala Ala Arg Arg Leu Ala Lys Cys Val Gln Tyr Gln Gly
 100 105 110
 Ala Ala Thr Val Glu Tyr Leu Tyr Ser Met Glu Thr Gly Glu Tyr Tyr
 115 120 125
 Phe Leu Glu Leu Asn Pro Arg Leu Gln Val Glu His Pro Val Thr Glu
 130 135 140
 Trp Ile Ala Glu Ile Asn Leu Pro Ala Ser Gln Val Val Val Gly Met
 145 150 155 160
 Gly Ile Pro Leu Tyr Asn Ile Pro Glu Ile Arg Arg Phe Tyr Gly Ile
 165 170 175
 Glu His Gly Gly Gly Tyr His Ala Trp Lys Glu Ile Ser Ala Val Ala
 180 185 190
 Thr Lys Phe Asp Leu Asp Lys Ala Gln Ser Val Lys Pro Lys Gly His
 195 200 205
 Cys Val Ala Val Arg Val Thr Ser Glu Asp Pro Asp Asp Gly Phe Lys
 210 215 220
 Pro Thr Ser Gly Arg Val Glu Glu Leu Asn Phe Lys Ser Lys Pro Asn
 225 230 235 240
 Val Trp Ala Tyr Phe Ser Val Xaa Ser Gly Gly Ala Ile His Glu Phe
 245 250 255
 Ser Asp Ser Gln Phe Gly His Val Phe Ala Xaa Gly Glu Ser Arg Ser
 260 265 270
 Leu Ala Ile Ala Asn Met Val Leu Gly Leu Lys Glu Ile Gln Ile Arg
 275 280 285
 Gly Glu Ile Arg Thr Asn Val Asp Tyr Thr Val Asp Leu Leu Asn Ala
 290 295 300
 Ala Glu Tyr Arg Glu Asn Xaa Ile His Thr Gly Trp Leu Asp Ser Arg
 305 310 315 320
 Ile Ala Met Arg Val Arg Ala Glu Arg Pro Pro Trp Tyr Leu Ser Val
 325 330 335
 Val Gly Gly Ala Leu Tyr Glu Ala Ser Ser Arg Ser Ser Ser Val Val
 340 345 350
 Thr Asp Tyr Val Gly Tyr Leu Ser Lys Gly Gln Ile Pro Pro Lys His
 355 360 365
 Ile Ser Leu Val Asn Leu Thr Val Thr Leu Asn Ile Asp Gly Ser Lys
 370 375 380
 Tyr Thr Ile Glu Thr Val Arg Gly Gly Pro Arg Ser Tyr Lys Leu Arg
 385 390 395 400
 Ile Asn Glu Ser Glu Val Glu Ala Glu Ile His Xaa Leu Arg Asp Gly
 405 410 415

113

Gly Xaa Leu Met Gln Xaa Asp Gly Asn Ser His Val Ile Tyr Ala Glu
 420 425 430
 Thr Glu Ala Xaa Gly Thr Arg Leu Leu Ile Asn Gly Arg Thr Cys Leu
 435 440 445
 Leu Gln Lys Glu His Asp Pro Ser Arg Leu Leu Ala Asp Thr Pro Cys
 450 455 460
 Lys Leu Leu Arg Phe Leu Val Ala Asp Xaa Ser His Val Val Ala Asp
 465 470 475 480
 Thr Pro Tyr Ala Glu Val Glu Ala Met Lys Met
 485 490

(2) INFORMATION FOR SEQ ID NO:110:

(i) Sequence characteristics:

(A) Length: 436 base pairs
 (B) Type: Nucleic acid
 (C) Strandedness: Single
 (D) Topology: Linear

(ii) Molecule type: Oligonucleotide

(xi) Sequence Description: SEQ ID NO:110:

TCTAGACTTT AACGAGATTC GTCAACTGCT GACAACTATT GCACAAACAG ATATCGCGGA 60
 AGTAACGCTC AAAAGTGATG ATTTTGAAC T AACGGTGCCT AAAGCTGTTG GTGTGAATAA 120
 TAGTGTGTG CCGGTTGTGA CAGCACCTT GAGTGGTGTG GTAGGTCGG GATTGCCATC 180
 GGCTATACCG ATTGTAGCCC ATGCTGCCCA ATCTCCATCT CCAGAGCCGG GAACAAGCCG 240
 TGCTGCTGAT CATGCTGTCA CGAGTTCTGG CTCACAGCCA GGAGCAAAA TCATTGACCA 300
 AAAATTAGCA GAAGTGGCTT CCCCAATGGT GGGAACATTT TACCGCGCTC CTGCACCAGG 360
 TGAAGCGGTA TTTGTGGAAG TCGGCGATCG CATCCGTCAA GGTCAAACCG TCTGCATCAT 420
 CGAAGCGATG AAAAUG 436

115

(2) INFORMATION FOR SEQ ID NO:112:**(i) Sequence characteristics:**

(A) Length: 22 base units
(B) Type: Nucleic acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Oligonucleotide**(ix) Features**

(A) NAME/KEY: N
(B) LOCATION: 11, 14
(C) IDENTIFICATION METHOD: N = A, G, C, T

(xi) Sequence Description: SEQ ID NO:112:

TCGAATTCGT NATNATHAAR GC

22

(2) INFORMATION FOR SEQ ID NO:113:**(i) Sequence characteristics:**

(A) Length: 22 base pairs
(B) Type: Nucleic acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Oligonucleotide**(ix) Features**

(A) NAME/KEY: N
(B) LOCATION: 17
(C) IDENTIFICATION METHOD: N = A, G, C, T

(xi) Sequence Description: SEQ ID NO:113:

GCTCTAGAGK RTGYTCNACY TC

22

(2) INFORMATION FOR SEQ ID NO:114:**(i) Sequence characteristics:**

(A) Length: 21 base pairs
(B) Type: Nucleic acid
(C) Strandedness: Single
(D) Topology: Linear

(ii) Molecule type: Oligonucleotide**(xi) Sequence Description: SEQ ID NO:114:**

GCTCTAGAAT ACTATTCCT G

21

116

(2) INFORMATION FOR SEQ ID NO:115:

(i) Sequence characteristics:

- (A) Length: 22 base pairs
- (B) Type: Nucleic acid
- (C) Strandedness: Single
- (D) Topology: Linear

(ii) Molecule type: Oligonucleotide

(ix) Features

- (A) NAME/KEY: N
- (B) LOCATION: 10, 20
- (C) IDENTIFICATION METHOD: N = A, G, C, T

(xi) Sequence Description: SEQ ID NO:115:

TCGAATTCWN CATYTTCATN RC

22

(2) INFORMATION FOR SEQ ID NO:116:

(i) Sequence characteristics:

- (A) Length: 23 base pairs
- (B) Type: Nucleic acid
- (C) Strandedness: Single
- (D) Topology: Linear

(ii) Molecule type: Oligonucleotide

(xi) Sequence Description: SEQ ID NO:116:

GCTCTAGAYT TYAAYGARAT HMG

23

-117-

WHAT IS CLAIMED IS:

1. An isolated and purified polynucleotide of from about 1350 to about 40,000 base pairs that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium.
2. The polynucleotide according to claim 1 wherein said polypeptide is a subunit of acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA.
3. The polynucleotide according to claim 1 wherein said cyanobacterium is *Anabaena* or *Synechococcus*.
4. The polynucleotide according to claim 3 wherein said biotin carboxyl carrier protein includes the amino acid residue sequence shown in SEQ ID NO:111 or a functional equivalent thereof.
5. The polynucleotide according to claim 1 wherein said polypeptide has the amino acid residue sequence of Figure 1 or Figure 2.
6. The polynucleotide according to claim 1 that includes (a) the DNA sequence of SEQ ID NO:1 from about nucleotide position 1300 to about nucleotide position 2650; (b) the DNA sequence of SEQ ID NO:1; or (c) the DNA sequence of SEQ ID NO:5.
7. An isolated and purified polynucleotide of from about 480 to about 40,000 base pairs that encodes a biotin carboxyl carrier protein of a cyanobacterium.
8. The polynucleotide according to claim 7 wherein said cyanobacterium is *Anabaena*.

-118-

9. The polynucleotide according to claim 8 wherein said biotin carboxyl carrier protein includes the amino acid residue sequence of SEQ ID NO:111 or a functional equivalent thereof.

10. The polynucleotide according to claim 7 that includes the DNA sequence of SEQ ID NO:110.

11. An isolated and purified DNA molecule comprising a promoter operatively linked to a coding region that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium, which coding region is operatively linked to a transcription-terminating region, whereby said promoter drives the transcription of said coding region.

12. An isolated and purified DNA molecule comprising a promoter operatively linked to an coding region that encodes a biotin carboxyl carrier protein of a cyanobacterium.

13. An isolated and purified polynucleotide of from about 1500 to about 150,000 base pairs that encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA.

14. The polynucleotide according to claim 13 wherein said plant polypeptide is a monocotyledonous plant polypeptide.

15. The polynucleotide according to claim 14 wherein said monocotyledonous plant is wheat, rice, maize, barley, rye, oats or timothy grass.

16. The polynucleotide according to claim 13 wherein said plant polypeptide is a dicotyledonous plant polypeptide.

-119-

17. The polynucleotide according to claim 16 wherein said dicotyledonous plant is soybean, rape, sunflower, tobacco, Arabidopsis, petunia, canola, pea, bean, tomato, potato, lettuce, spinach, carrot, canola, alfalfa, or cotton.

18. The polynucleotide according to claim 13 wherein said plant polypeptide includes the amino acid residue sequence of SEQ ID NO:109.

19. The polynucleotide according to claim 13 that includes the nucleotide sequence of SEQ ID NO:7.

20. An isolated polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium.

21. The polypeptide according to claim 20 wherein said cyanobacterium is *Anabaena* or *Synechococcus*.

22. The polypeptide according to claim 20 wherein said biotin carboxyl carrier protein includes the amino acid sequence of SEQ ID NO:111.

23. The polypeptide according to claim 20 having the amino acid residue sequence of Figure 1 or Figure 2.

24. An isolated and purified biotin carboxyl carrier protein of a cyanobacterium.

25. The protein according to claim 24 wherein said cyanobacterium is *Anabaena*.

-120-

26. The protein according to claim 25 including the amino acid residue sequence of SEQ ID NO:111.

27. An isolated and purified plant polypeptide having a molecular weight of about 220 KD, dimers of which have the ability to catalyze the carboxylation of acetyl-CoA.

28. A process of increasing the herbicide resistance of a monocotyledonous plant comprising transforming said plant with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby said promoter is capable of driving the transcription of said coding region in a monocotyledonous plant.

29. The process according to claim 28 wherein said polypeptide is an acetyl-CoA carboxylase enzyme.

30. The process according to claim 29 wherein said acetyl-CoA carboxylase is a dicotyledonous plant acetyl-CoA carboxylase enzyme.

31. The process according to claim 30 wherein said coding region includes the DNA sequence of SEQ ID NO:108.

32. The process according to claim 28 wherein said promoter is CaMV35.

33. A transformed plant produced in accordance with the process of claim 28.

-121-

34. A transgenic plant having incorporated into its genome a transgene that encodes a dicotyledonous polypeptide having the ability to catalyze the carboxylation of acetyl-CoA.

35. A process of altering the carboxylation of acetyl-CoA in a cell comprising transforming said cell with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby said promoter is capable of driving the transcription of said coding region in said cell.

36. The process according to claim 35 wherein said cell is a cyanobacterium or a plant cell.

37. The process according to claim 35 wherein said plant polypeptide is a plant acetyl-CoA carboxylase enzyme.

38. The process according to claim 37 wherein said plant acetyl-CoA carboxylase enzyme is a monocotyledonous plant acetyl-CoA carboxylase enzyme.

39. The process according to claim 38 wherein said monocotyledonous plant acetyl-CoA carboxylase enzyme is wheat acetyl-CoA carboxylase enzyme.

40. A transformed cyanobacterium produced in accordance with the process of claim 36.

41. A process for determining the inheritance of plant resistance to herbicides of the aryloxyphenocyclopropanate or cyclohexanedione class, which process comprises the steps of:

-122-

- (a) measuring resistance to herbicides of the aryloxyphenocyclopropanate or cyclohexanedione class in a parental plant line and in progeny of said parental plant line;
- (b) purifying DNA from said parental plant line and said progeny;
- (c) digesting said DNA with restriction enzymes to form DNA fragments;
- (d) fractionating said fragments on a gel;
- (e) transferring said fragments to a filter support;
- (f) annealing said fragments with a labelled RFLP probe consisting of a DNA molecule that encodes acetyl-CoA carboxylase or a portion thereof; and
- (g) detecting the presence of complexes between said fragments and said RFLP probe; and
- (h) correlating the herbicide resistance of step (a) with the complexes of step (g) and thereby the inheritance of herbicide resistance.

42. The process according to claim 41 wherein said acetyl-CoA carboxylase is a dicotyledonous plant acetyl-CoA carboxylase enzyme.

43. The process according to claim 41 wherein said acetyl-CoA carboxylase is a mutated monocotyledonous plant acetyl-CoA carboxylase that confers herbicide resistance or a hybrid acetyl-CoA carboxylase comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase, a portion of a dicotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

44. A process for identifying herbicide resistant variants of a plant acetyl-CoA carboxylase comprising the steps of:

- (a) transforming cyanobacteria with a DNA molecule that encodes a monocotyledonous plant acetyl-CoA carboxylase enzyme to form transformed cyanobacteria;

-123-

- (b) inactivating cyanobacterial acetyl-CoA carboxylase;
- (c) exposing said transformed cyanobacteria to a herbicide that inhibits acetyl-CoA carboxylase activity;
- (d) identifying transformed cyanobacteria that are resistant to said herbicide; and
- (e) characterizing DNA that encodes acetyl-CoA carboxylase from the cyanobacteria of step (d).

AAGCTTTTATATTTTCCATTCTAGAACTTAGCTGCATCGGCCCAAGTATTTTGTCAAATATGGCGAAAAGACTTCATAAATCAAGGT 90
 TAAAGGTTGACCGTGATGCCAAAACAGGTAATGGCGACCCAGAAAGGCCCATCCACGCCAAAACCTAATTGCAAGGCCCTCTGAATTTCC 180
 GTAATAAATACCCGACATCCCGATACAACCTCCGTGGGAAGACGAGCTAGACTTGGCCAAATTTGGTAATGAACGGTTTTGCAAAATCTC 270
 GTCTACATGGCTGGCTTCCCAACCATGAGGTTGCATAGGGGAGTCGTGGCCAGAGCGGTACGTAGCCATACCTGTCCGCCGAGCTCTTGG 360
 CGCTGGAAACAGATTGGATTAAATCCGGCGCACTATCTAAATCCAAACCAATCAATGACATATCAATGACATCGACTTCTGTTGGCTCACC 450
 AGTAAGTAAATCTAAATGCCTTGTGGGTGAGCCATCACCTAAGAGTAGTAGTTGCCACGCTGGAGCCAGCTGAGTGTGAGGCAAACTATG 540
 TTTAATTACTTCTTCCCCACCTTGCCAAATAGGAGTGAGGCGATGCCATCCGCTGGCAGTGTGAGTGTGCTTGGAGTAAAAGTGGC 630
 AGTCAATGTTCTTTACAAAAGTTCACCTATTTATATCAAAGCATAAAAAATTAAATAGTTGTGAGTTGTGATTTGGTTATCTCTTTTGTCT 720
 CCCCCTGCCCTTACTTCCCTCCTCTGCCCCAATAATTAGAAAGGTGAGGAGTCAAAAACCTATCACTTTTGACCACTGACCTTTCACAAT 810
 TGACTATAGTCACTAAAAAATGGCGATGGCGAGACTCGAAGTGGCAAGGCCACACGCCCTCAAGCGTGGCGGTATACCAATTC 900
 GCCACATCCGCAAGGTTGTACAAGAAGATATACTAGCACAAAAAATTGCATAAAACAAGGTAAAACCTATATTTGCCAAACTTTATGGA 990
 AAATTTATCTTGTCTAAATATACAAATTTCCCGAAGAGGATACGAGACTAACAGAAATGTAGTATCGCCACAAGTGATATTAAGGGGGTA 1080
 TGGGGTTTCTTCCCTTACACCTTAAACCTTCACACCCACCTCCATGAAAAATCTTGTGGTAAGTCCGTTTCTGCAATTTATTTA 1170

AAGATGAGCCTGGGTATCTCCTGTCATAATTGAGATGAAGCGATGCTAAGCGGCTACGCTACGCGCTAAAAGCAACTTGGATGGGA 1260
 * D E A M P K A A T L R A K S N L D G R
 GACAATTTCTATCTGCTGACTGATCTGATATCGAAAACCTAGAAAATGAAGTTTGACAAAATATTAATTGCCAATCGGGGAGAAATAG 1350
 Q F L S A G T D T D I E N * K M K F D K I L I A N R G E I A
 CGCTGGCAATCTCCGCGCTGTGAGGAAATGGGATTCGAGCATTCGAGTGTGACCGGAATGCTCTTCATGTCACAAC 1440
 L R I L R A C E E M G I A T I A V H S T V D R N A L H V Q L
 TTGCTGACGAAGCGGTTTGTATGGCGAACCTGCTAGCGCTAAAAGTTTGAATATTTCCCAATATTATGTCGGCGCTTAAACGGCA 1530
 A D E A V C I G E P A S A K S Y L N I P N I I A A L T R N
 ATGCGAGTCTATCTCTGGGTATGGCTTTTATCTGAAAATGCCAAATTTGCGGAAATCTGTGCTGACCATCACATTGCATTCTTG 1620
 A S A I H P G Y G F L S E N A K F A E I C A D H I A F I G
 GCCCCACCCAGAGCTATCCGCTCATGGGGACAAATCCACTGCCAAGGAAACCATGCAAAAAGCTGGTGTACCGACAGTACCGGGTA 1710
 P T P E A I R L M G D K S T A K E T M O K A G V P T V P G S
 GTGAAGGTTTGGTAGACAGAGCAAGAAGATTAGAAGTGGCGAAGATATTGGCTACCCAGTGATGATCAAAGCCACGGCTGGTGGT 1800
 E G L V E T E Q E G L E L A K D I G Y P V M I K A T A G G G
 GCGGCGGGGTATGCGACTGGTGGATCGCCAGATGAATTTGTCAAAGTGTCTTAGCCGCCCAAGGTGAAGCTGGTGCGAGCCTTTGGTA 1890
 G R G M R L V R S P D E F V K L F L A A Q G E A G A A F G N
 ATGCTGGCGTTTATATAGAAAAATTTATGAACGTCCGCGCCACATTTGAATTTCAAATTTTGGCTGATAATTACGGCAATGTGATTCACT 1980
 A G V Y I E K F I E R P R H I E F Q I L A D N Y G N V I H L
 TGGGTGAGAGGGATTGCTCAATTCAGCGTCTGAACAAAAGTTACTAGAAGAAGCCCCAGCCAGCCTTGGACTCAGACCTAAGGGAAA 2070
 G E R D C S I Q R R N Q K L L E E A P S P A L D S D L R E K
 AAATGGGACAAGCGCGGTGAAGCGGCTCAGTTTATCAATTACGCCGGGCGAGTACTATCGAGTTTGTGCTAGATAGATCCGGTCACT 2160
 M G Q A A V K A A Q F I N Y A G A G T I E F L L D R S G Q F
 TTTACTTTATGAGATGAACACCCGGATTCAAGTAGAACATCCCGTAAGTGAAGTGTGAGTGGATTATTTGGTGTAGCAATCA 2250
 Y F M E M N T R I Q V E H P V T E M V T G V D L L V E Q I R
 GAATTCGCCAAGGGGAAGACTTAGACTAACTCAAGACCAAGTAGTTTACGCCGTATGCGATCGAATGTGCGATCAATGCCGAAGACC 2340
 I A Q G E R L R L T Q D Q V L R G H A I E C R I N A E D P
 CAGACCAGGATTTCGCCCGCAGCCCGGACGATTAGCGGTTATCTTCCCGTGGCGGCTGGCGTGGGATTGACTCCCAGCTTTACA 2430
 D H D F R P A P G R I S G Y L P P G G P G V R I D S H V Y T
 CGGATTACCAAAATCCGCGCTTACTAGGATTCTTAATTTGGTAATGTAGCTTTGGGGCCCTGATCGCGCTACTGCTATTAACCGCATGA 2520
 D Y Q I P P Y Y D S L I G K L I V W G P D R A T A I N R M X
 AACCGCGGCTCAGGGAATGCGCCATCACTGATTACCTACAACCATTTGGGTTTCATCAAAGAATTATGGAATAATCCCAATTTTACAAG 2610
 R A L R E C A I T G L P T I G F H Q R I M E N P Q F L Q G
 GTAATGTGTCTACTAGTTTGTGCGAGGAGATGAATAAATAGGTAATGGGTAATGGGTAATAGAGTTTCAATCACCATTACC 2700
 N V S T S F V Q E M N K * * W V M G N R V S I T N Y Q
 AATCCCTAATCTCCGTGCCAATCTGTCAGTAATCTTGTGCGCTAGAAGAATCTTCGCAACAGGCTAAAATACCAACACACAC 2790
 F P N S V P T S S V I L A G L E E L L A T G *

AATGGGGGTGATATCAACACCACCTATTGGTGGGATGATTTTTCGCAAGGGAATGAGAAATGGTTCACTCGGCCAAGCAATTAAGTTGAA 2880
 GGGCAACGGTTTCAGATCGACTTGGGATACCAAGTCAAGATATACGGAATAAACAAGAAATGTATCATCTCCCAATACAGGGGCCAAG 2970
 AATCCAAACGCTCAGGTTAACACCAAGTATCGATCTAAGCTACTATTTTGTGAATTTACAAAAAAGCTGCAAGCAAAAGCTGAAATTTTA 3060
 AGCTT

FIG. 1

2/9

ATGCGTTTCA ACAAGATCCT GATCGCCAAT CGCGGCGAAA TOGCOCTGOG CATTCTOOGC
 ACTTGTGAAG AACTCGGGAT OGGCAGGATC GCCGTTCACT CCCTGTGGA TOGCAOOGC
 CTCCATGTGC AGTTAGCGGA CGAAGCGGTC TGTATTGGOG AAGOGGOCAG CAGCAAAAGC
 TATCTCAATA TCCCCAACAT CATTGCGGCG GCCCTGACCC GGAATGOCAG CGOCATTAC
 CCCGGCTATG GCTTCMTGGC GGAGAATGCC CGCTTTGCAG AAATCTGOGC CGATCAOCAT
 CTCACCTTTA TTGGCCCCAG CCGGATTCG ATTOGAGCCA TGGGOGATAA ATCCACOGCT
 AAGGAAACAA TGCAGCGGGT CGGCGTCCG ACGATTCCGG GCASTGAOCG TCTGCTGACG
 GATGTTGATT CGGCTGCCAA AGTTGCTGCC GAGATCGGCT ATCCOOGTCAT GATCAAAGCG
 ACGGCGGGGG GCGGTGGTCG CGGATGCGG CTGGTGGGTG AGOCTGCAGA TCTGGA AAAA
 CTGTCCTTTG CTGCCCAAGG AGAAGCCGAG GCAGCTTTTG GGAATOCAGG ACTGTATCTC
 GAAAAATTTA TOGATCGCCC ACGCCACGTT GAATTTGAGA TCTTGGCOGA TGCTAOGGC
 AATGTAGTGC ATCTAGGCGA GCGCGATTGC TCCATTCAAC GTCGTCACCA AAAGCTGCTC
 GAAGAAGCCC CCAGTCCGGC GCTATCGGCA GACCTGCGGC AGAATGAGG CGATGCGGCC
 GTCAAAGTCG CTCAAGCGAT CGGCTACATC GGTGCCGGCA CCGTGGAGTT TCTGGTGGAT
 GCGACCGGCA ACTTCTACTT CATGGAGATG AATACCGCA TCCAGTGA GCATCCAGTC
 ACAGAAATGA TTACGGGACT GGACTTGATT GCGGAGCAGA TTCGGATTGC CCAAGGCGAA
 GCGCTGCGCT TCCGGCAAGC CGATATTCAA CTGCGCGGCC ATGGGATGA ATGCGGTATC
 AATGCGGAAG ATCCGGAATA CAATTTCCGG CCGAATCCTG GCGGCATTAC AGGCTATTTA
 CCGCCCGGGG GCGCGGGCGT TCGTGTGGAT TCCCATGTTT ATACCGACTA CGAAATTCGG
 CCGTATTACG ATTGCTGAT TGGCAAAATG ATTGCTGCGG GTGCAACACG GGAAGAGCGG
 ATCGCGCGGA TGCAGCGTGC TCTGCGGGAA TCGGCCATCA CCGGCTTGCC GACGACCTT
 AGTTTCCATC AGCTGATGTT GCAGATGCCT GAGTCCCTGC GCGGGGAAT CTATACCAAC
 TTTGTTGAGC AGGTGATGCT ACCTCGGATC CTCAAGTCT AG

amino acid sequence

MRFNKILIAN RGEIALRILR TCEELGIGTI AVHSTVDRNA LHVQLADEAV CIGEAASSKS
 YLNIPNIIAA ALTRNASATH PGYGLAENA REAEICADHH LTFLEPSPDS IRAMGDKSTA
 KETMQRVGVP TIPGSDGLLT DVDSAAKVAA EIGYPVMIKA TAGGGGRGMR LVREPADLEK
 LFLAAQGEAE AAFGNPGLYL EKFDPRPRHV EFQILADAYG NVVELGERDC SIQRRHQKLL
 EEAPSPALSA ELRQKMDAA VKVAQAIGYI GAGTVEFLVD ATGTFYFEM NTRIQVEHPV
 TEMITGLDLI AEQIRIAQGE ALRFQADIQ LRGHATECRI NAEDPEYNFR PNPGRITGYL
 PPGGPGVRVD SHVYTDYEIP PYYDSLIGKL IHWGATREEA IARMQALRE CAITGLPTTL
 SFHQMLQMP EFLRGELYTN FVEQVMLPRI LKS

FIG. 2

3/9

```

Wh ACC ..... 100
Rt ACC MDEPSPLAKTLELNOHSRF IIGSVSEDNSEDEIS-NLVKLDLEEKGLSPASVSDTISDLGISAQDGLAFHMRSSMSGLHLVKQGRDRKKIDSQRDF
Ch ACC MEESQPAKPLEMNPHSRF IIGSVSEDNSEDETSSLVKLDLLEEKERSLSPVSCSDSLDGLPSAQDGLANHMRPSMSGLHLVKQGRDRKKVDVQRDF
Yt ACC MSESLEFPSPQKMEYEITNYSERHTELPGHF IGLNTVDKL
Sy ACC
An ACC
Ee ACC
Hm PCCA
Rt PCCA
Yt PC

MLSAALRTLKHVLYYSRQCL
MPYRERFCAIRWCNRSGRSSQQLLWTLKRAPVYSQCL
MS

Wh ACC ..... 200
Rt ACC TVASPAEFVTRFGGNVIEKVL IANNGIAAVKCMRS IRRWSYEMFRNERA IREFVMVTPEDLKANA EYIRKADHYVPVPGGANNNNYANVEL ILDIAKR
Ch ACC TVASPAEFVTRFGGNVIEKVL IANNGIAAVKCMRS IRRWSYEMFRNERA IREFVMVTPEDLKANA EYIRKADHYVPVPGGANNNNYANVEL ILDIAKR
Yt ACC EESPLRDFVKSHGGHTVI SKILLIANNGIAAVKE IIRSVRKWAYE IFGDDRTVOFVAMATPEDLEANA EYIRKADQYIEVPGGTNNNNYANVLDIVDIAER
Sy ACC MRFNKILIANRGEIALRLRLTCEELGIGTIAVHSTVD--RNALHVQLADEAVCIGEAASS-----KSYLNIPNIIAAALT
An ACC MKFDKILIANRGEIALRLRACEEMGIATIAVHSTVD--RNALHVQLADEAVCIGEPASA-----KSYLNIPNIIAAALT
Ee ACC MLDKIVIANRGEIALRLRLRACKELGIRTVAVHSSAD--RDLKHVLLADETVCIGEPAPSV-----KSYLNIPATISAAEI
Hm PCCA MVSRLNLSVGVDPNKIFDKILVANRGEIACRVIRCTCKRMG IKTVAIHSDVD--ASSVHVKMADEAVCVGPAPTS-----KSYLNMDAIMEAIKK
Rt PCCA VVSRLSLSVEYEPKEKIFDKILIANRGEIACRVIKTRKMG IRTVAIHSDVD--ASSVHVKMADEAVCVGPAPTS-----KSYLNMDAIMEAIKK
Yt PC QRFAGLRDNFNLLGEK-NKILVANRGEIPIRIFRTAHELMSQTVATYSHED--RLSTHKQKADEAYVIGEVGYTPV-----GAYLAIDEIISIAOK

Wh ACC ..... 300
Rt ACC IPVQAVWAGWGHASENPKLP ELL--LKNGLAFMGPPSOAMWALGDKIASSIVAQTAGIPTLPWNGSGLRVDWQENDESKRI LNVQDLYEKG YVKDADD
Ch ACC IPVQAVWAGWGHASENPKLP ELL--HKNGIAFMGPPSOAMWALGDKIASSIVAQTAGIPTLPWNGSGLRVDWQENDESKRI LNVQDLYEKG YVKDADD
Yt ACC ADVDAVWAGWGHASENPKLP ELL--LKNGLAFMGPPSOAMWALGDKIASSIVAQTAGIPTLPWNGSGLRVDWQENDESKRI LNVQDLYEKG YVKDADD
Sy ACC RNASAIHPGYGFLSENARFAE IC--ADHHLTF IGPSPDSIRAMGDKSTAKETMORVGVPTIPGSDG-L-----LTDVDS
An ACC RNASAIHPGYGFLSENARFAE IC--ADHHLTF IGPSPDSIRAMGDKSTAKETMORVGVPTIPGSDG-L-----LTDVDS
Ee ACC TGAVAIHPGYGFLSENARFAE IC--ADHHLTF IGPSPDSIRAMGDKSTAKETMORVGVPTIPGSDG-L-----LTDVDS
Hm PCCA TRAQAVHPGYGFLSENARFAE IC--ADHHLTF IGPSPDSIRAMGDKSTAKETMORVGVPTIPGSDG-L-----LTDVDS
Rt PCCA TRAQAVHPGYGFLSENARFAE IC--ADHHLTF IGPSPDSIRAMGDKSTAKETMORVGVPTIPGSDG-L-----LTDVDS
Yt PC HQVDFIHPGYGFLSENARFAE IC--ADHHLTF IGPSPDSIRAMGDKSTAKETMORVGVPTIPGSDG-L-----LTDVDS

Wh ACC ..... 400
Rt ACC .....VMIKASWGGGGRGIRKVNNDDEVRALFQVQGEVPGS-----P I FIMKVASQSRHLEVQLLCKHGNVAALHSRDCSVORRHQKIIEEG
Ch ACC GLKAAEEVGYPMIKASEGGGGGIRKVNNDDEVRALFQVQGEVPGS-----P I FIMKVASQSRHLEVQLLCKHGNVAALHSRDCSVORRHQKIIEEG
Yt ACC GLRAAEVGYPMIKASEGGGGGIRKVNNDDEVRALFQVQGEVPGS-----P I FIMKVASQSRHLEVQLLCKHGNVAALHSRDCSVORRHQKIIEEG
Sy ACC GLQAKRIGFPMIKASEGGGGGIRKVNNDDEVRALFQVQGEVPGS-----P I FIMKVASQSRHLEVQLLCKHGNVAALHSRDCSVORRHQKIIEEG
An ACC AAKVAE IGYPMIKATAGGGGGRMRLVREPADLEKFLAAGGAEAAFGNGLYLEKF IERPRHVEFQILADAGYGNVHLGERDCS IORRHQKLEEA
Ee ACC GLELAKDIGYPMIKATAGGGGGRMRLVREPADLEKFLAAGGAEAAFGNGLYLEKF IERPRHVEFQILADAGYGNVHLGERDCS IORRHQKLEEA
Hm PCCA NRIAKRIGYPMIKATAGGGGGRMRLVREPADLEKFLAAGGAEAAFGNGLYLEKF IERPRHVEFQILADAGYGNVHLGERDCS IORRHQKLEEA
Rt PCCA AVRIARE IGYPMIKASAGGGGGRMRLVREPADLEKFLAAGGAEAAFGNGLYLEKF IERPRHVEFQILADAGYGNVHLGERDCS IORRHQKLEEA
Yt PC ALDFVNEYGYPMIKATAGGGGGRMRLVREPADLEKFLAAGGAEAAFGNGLYLEKF IERPRHVEFQILADAGYGNVHLGERDCS IORRHQKLEEA

Wh ACC ..... 500
Rt ACC PITVAPPETIKELEQAARRLAKCVQYQGAATVEYL YSMETGEYFLELNPRLQVEHPVTEWIAE INLPASOVVVGMIPLYNPIE IRRFYGIEHGGGYH
Ch ACC PAATATPAVEFHEMEQCAVKLAKMVGYSAGTVEYL YSQD-GSFYFLELNPRLQVEHPCTEMVADVNLPAAQLQIANGIPLFR IRIKIRMYGVSPWGDAP
Yt ACC PASIATSVFHEMEQCAVKLAKMVGYSAGTVEYL YSQD-GSFYFLELNPRLQVEHPCTEMVADVNLPAAQLQIANGIPLFR IRIKIRMYGVSPWGDAP
Sy ACC PVTIAKAEIHEMEKAARVRLGLVGYVYAGTVEYL YSHDDGKFYFLELNPRLQVEHPCTEMVADVNLPAAQLQIANGIPLFR IRIKIRMYGVSPWGDAP
An ACC PSPALSADLRQMGDAAVKVAQAGYIGAGTVEFLVD -ATGNFYFEMMNTRIQVEHPVTEWIAE INLPASOVVVGMIPLYNPIE IRRFYGIEHGGGYH
Ee ACC PSPALSADLRQMGDAAVKVAQAGYIGAGTVEFLVD -ATGNFYFEMMNTRIQVEHPVTEWIAE INLPASOVVVGMIPLYNPIE IRRFYGIEHGGGYH
Hm PCCA PAPGITPELRRYIGERCAKACVDIGYRGAGTVEFLVD -ATGNFYFEMMNTRIQVEHPVTEWIAE INLPASOVVVGMIPLYNPIE IRRFYGIEHGGGYH
Rt PCCA PSIFLDAETRRAMEQAVALARAVYSSAGTVEFLVD SQ-KNFYFLELNPRLQVEHPVTEC IHWPGSPGKTVLQEHLSGTNKLIFA
Yt PC PARTLPREVRDAILTDVAVLAKCECGYRNAGTAEFLVDNQ-NRHYFIEINPRIQVEHTIIEEITGIDIVAAGIQAAGASLPQLGLFQDKIT

Wh ACC ..... 600
Rt ACC AWKEISAVATKFDLDKAGSVKPKGHCVAVRVTS EDDPDGFK-PTSGRVEELNEKSPKNVWAYF-----SVKSGGAIHEFSDSQGHVAFGESRSLAIAN
Ch ACC IDFENSAHVPC-----PRGHVIAARI TSENPDGFK-PTSGRVEELNEKSPKNVWAYF-----SVKSGGAIHEFSDSQGHVAFGESRSLAIAN
Yt ACC IDFENSAHVPC-----PRGHVIAARI TSENPDGFK-PTSGRVEELNEKSPKNVWAYF-----SVKSGGAIHEFSDSQGHVAFGESRSLAIAN
Sy ACC IDFEFTQDAT-----PKORRPIPKGHCTACRITSEDPNDGFK-PTSGRVEELNEKSPKNVWAYF-----SVKSGGAIHEFSDSQGHVAFGESRSLAIAN
An ACC -----LRGHAIECRINAEDPEYNE-RPNPGRITR-----YLPGGG-PGVRVDS-HVYTDYEIPPYDSLIGKLIWVGATREAAIAR
Ee ACC -----LRGHAIECRINAEDPEYNE-RPNPGRITR-----YLPGGG-PGVRVDS-HVYTDYEIPPYDSLIGKLIWVGATREAAIAR
Hm PCCA -----VRGHAIECRINAEDPEYNE-RPNPGRITR-----YLPGGG-PGVRVDS-HVYTDYEIPPYDSLIGKLIWVGATREAAIAR
Rt PCCA -----FNGWAVECRVYAEDEPKSFGSLPSIGRLSQ-----YQEP IHLPGVRVDS-GIOPGSDIS IYHDPMSIKLITYGSDRTEALKR
Yt PC -----ISGWAECRVYAEDEPKSFGSLPSIGRLSQ-----YQEP IHLPGVRVDS-GIOPGSDIS IYHDPMSIKLITYGSDRTEALKR

```

FIG. 3-1

```

Wh ACC  MVLGLKEIQIRGEIRTNVDYTVDLLNAAEYRENMIHTGWLDRIAMRVRAERPPWYLSVVGALYEASSRSSSVVTDYVGYLSKGQIPPK----- 700
Rt ACC  MVLVALKELSIKRGDFRTTVEYLIKLETESFQINRIDTGWLDRIAEKVQAEKPDMLGVVCGALHVADVNLRNSISNLFHSLERGOVLPA-----
Ch ACC  MVLVALKELSIKRGDFRTTVEYLIKLETESFQINRIDTGWLDRIAEKVQAEKPDMLGVVCGALHVADVSFRNSVSNFLHSLERGOVLPA-----
Yt ACC  MVLVALKELSIKRGDFRTTVEYLIKLETESFQINRIDTGWLDRIAEKVQAEKPDMLGVVCGALHVADVSFRNSVSNFLHSLERGOVLPA-----
Sy ACC  MQRALRECAITG-LPTTISFHQIMQMPFIRGELYTNFVEQVMLPRIKS
An ACC  MKRALRECAITG-LPTTISFHQIRIMENPOFLQGNVSTSFVQEMNK
Ec ACC  MKNALQELIDG-IKTNVDLQIRIMNDENFOHGGTNIHYLEKKLGLOEK
Hm PCCA  MADALDNYVIRG-VTHNIPLLREVIINSREVKGDISTKFLSDVYPDGFKGHMLTPSERDOLLAIASSLFVASQLRAQRFQEHRSVPVIRPDVAKWELSV
Rt PCCA  MEDALDSYVIRG-VTHNIPLLREVIINTRFVKGDISTKFLSDVYPDGFKGHMLTPSERDOLLAIASSLFVASQLRAQRFQEHRSVPVIRPDVAKWELSV
Yt PC    MIRALIEFRIRG-VKTNIPFLLTLLTNPFIEGTWYGTIDDTQPLQFQMVSSQNRAQKLLHYLADVADNGSSIKGOIGLPKLSNPSVPH--SYNMP
Kp ODA
PS TC
* * * *

Wh ACC  -----HISLVNLTVTNLIDGSKYTIETVRGGPRS YKLRINESEVEAEIHLRDGGLLMQLDGNSHVIAETEAAGTRLLINGRTCLLOKEHDP SRL 800
Rt ACC  -----HTLLNTVDVELIYEGIKYVLKVTQSPNSYVIMNGSCVEVDVHRLSDGGLLLSYDGSSYTTMKEEVDORYRITIGNKTCVFEKENDPSVM
Ch ACC  -----HTLLNTVDVELIYEGIKYVLKVTQSPNSYVIMNGSCVEVDVHRLSDGGLLLSYDGSSYTTMKEEVDORYRITIGNKTCVFEKENDPSVM
Yt ACC  -----DLQTMFPVDFIHEGKRYKFTVAKSGNDRYTLFINGSKCDIILROLSDGGLLTAIGGKSHTIYWKEEVAATRLSVD SMTLLLEVENDPTOL
An ACC  TDIAEVLTKSDDFELTVRKAVGVNNSVVPVVTAPLSGVVSGSLPSAIP IVAHAAPSPSPEPGTSRAADHAVTSSGQPGAKIIDOKLAEVASPMVGTIFY
Ec ACC  MDIRKIKKLIELVEESGISELEISEGEESVRISRAAPAAEFVMOQAYAAPMMQOPAQSNAAAPATVPSMEAPAAAEISGHIVRSPMVGTFY
Hm PCCA  KLHDKVHTVVASNNGSVFSVEVDGSKLNVSTIWNLASPLLSVSVVDGTQRTVQCLSRAGGNSIQF LGTVYKVNILTRLAELNKFMLEKVTDTSVL
Rt PCCA  KLHDEHDTVVASNNGPTFNVEVDGSKLNVSTIWNLASPLLSVSVVDGTQRTVQCLSRAGGNSIQF LGTVYKVNILTRLAELNKFMLEKVTDTSVL
Yt PC    RVYEDFQKMETYGDLSVLPTRSFLSPLETDEEIEVVEOGKTLIKLQAVGDLNKKTGEREVYFDLNGEMRKIRVADRSQKVEVTYKSKADMHDPLHI
Kp ODA  VLTVALEFPQGLKFLNRHNPAAFEFVPQAEAAQPVAKAEKPAASGVYTVVEGKAFVVKVSDGGDVSQLTAAAPAPAPAPASAPAAAAAPAGAGTPV
PS TC    MKLKVTVNGTAYDVDVDVKSHENPMGTIILFGGGTGGAPAPRAAGGAGAGKAGEGEI
-----

Wh ACC  LADTPCKLRLFLVADGSHVADTPYAEVEAMQM..... 900
Rt ACC  RSPSAGKLIQYIVEDGGHVFAGOCYAEIEVMQMVMILTAVESGCIHYVRPGAALDPGCVIAKMLQDNPSKVQOAE LHIGSLPQIQSTALRGEKLRHIF
Ch ACC  RSPSAGKLIQYIVEDGGHVFAGOCYAEIEVMQMVMILTAVESGCIHYVRPGAALDPGCVIAKMLQDNPSKVQOAE LHIGSLPQIQSTALRGEKLRHIF
Yt ACC  RTPSPGKLVKFLVNGEHIKGOQYAEIEVMQMOMPLVSOENGIVQLLKQPGSTIVAGDIMAIMTLDDPSKVKHALPFEGMLPDFGSPVIEGTRPAYKF
An ACC  RAPAPGE--AVFVEVGDRIRQGTVC IIEAMKM.....
Ec ACC  RTPSPDA--KAFIEVGQKVNVDGTLICIVEAMQMOMQIEADKSGTVKAILVESGQPVFDEPLVVIE
Hm PCCA  RSPMPGVVAVSVKPGDVAEGQIEICVIEAMQMOMNSMTAGKTGTVKSVHCKAGDVTGEGDLLVELE
Rt PCCA  RSPKPGVVAVSVKPGDMVAEGQIEICVIEAMQMOMNSMTAGKMGKVLVHCKAGDVTGEGDLLVELE
Yt PC    GAPMAGVIVEVRHKGSLIKKQGPVAVLSAMKMEMIISPSDQGVKEVFSVDCENVDSSDLLVLLEDQVPVETKA
Kp ODA  TAPLAGTIWKLASEGOTVAGEVLLILEAMKMETEIRAAQAGTVRGIAVKAGDAVAVGDTLMTLA
PS TC    PAPLAGTVSKILVKEGDTVKAGQTVLVLEAMKMETEINAPTDGKVEKVLVKERDAVQGGQGLIKIG
-----

```

FIG. 3-2

Biotin carboxylase primers

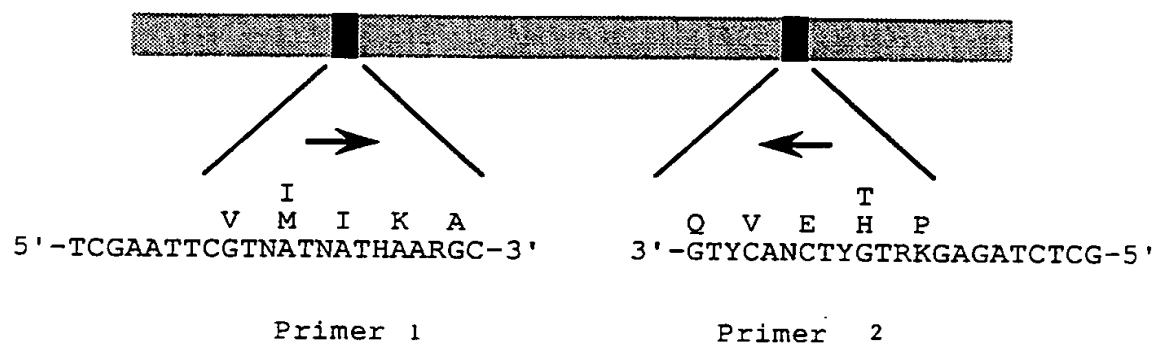


FIG. 4

Biotin carboxylase / biotin carboxyl carrier domain primers

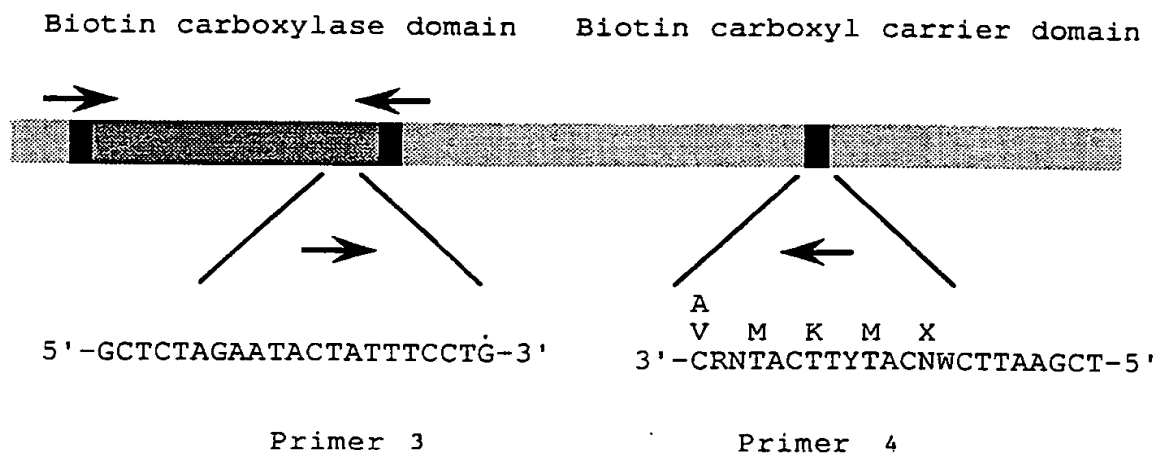


FIG. 5

GTGATGATCAAGGCATCATGGGGTGGGGTGGTAAAGGAATAAGGAAGGTACATAATGATGATGAGGTCAGAGCAITGTTTAAAGCAAGTG 90
 V M I K A S W G G G G K G I R K V H N D D E V R A L F K Q V
 CAAGGAGAAGTCCCGGATCGCCTATATTTATTTATGAAGGTGGCATCTCAGAGTCGACATCTAGAGGTTCAATTGCTCTGTGACAAGCAT 180
 Q G E V P G S P I F I M K V A S Q S R H L E V Q L L C D K H
 GGCAACGTGGCAGCACTGCACAGTCGAGACTGTAGTGTTCAAAGAAGGCATCAAAAGATCATTGAGGAGGGACCAATTACAGTTGCTCT 270
 G N V A A L H S R D C S V Q R R H Q K I I E E G P I T V A P
 CCAGAAACAATTAAAGAGCTTGAGCAGGCGGCAAGGCGACTAGCTAAATGTGTGCAATATCAGGGTCTGCTACAGTGGAAATATCTGTAC 360
 P E T I K E L E Q A A R R L A K C V Q Y Q G A A T V E Y L Y
 AGCATGGAAACAGGCGAATCTATTTCTGTGAGCTTAATCAAGGTTGCAAGTAGACACCTCTGACCGAATGGATTGCTGAATAAAC 450
 S M E T G E Y Y F L E L N P R L Q V E H P V T E W I A E I N
 C
 T
 TTACTGCACTCAAGTTGTAGTAGGAATGGGCATACCCTCTACAACATTCAGAGATCAGAGCGCTTTTATGGAATAGAACATGGAGGT 540
 L P A S Q V V V G M G I P L Y N I P E I R R F Y G I E H G G
 C C C G
 GGCTATCATGCTTGGAGGAATATCAGCTGTTCACATAAATTGATTGGACAAAGCAGCTCTGTAAAGCCAAAGGTCATTGTGTA 630
 G Y H A W K E I S A V A T K F D L D K A Q S V K P K G H C V
 A G
 CGAGTTAGAGTTACTAGCAGGATCCAGATGATGGGTTTAAAGCTACAGTGGAGAGTAGAAGAGCTGAACCTTAAAGTAAGCCCAAT 720
 A V R V T S E D P D D G F K P T S G R V E E L N F K S K P N
 C G C T
 GTTGGGCTTATTTCTCGTAACTCCGAGGTCGAATTCAGAGTTCTCTGATTCCAGTTTGGTCATGTTTTTCTTTTGGGGAATCT 810
 V W A Y F S V K S G G A I H E F S D S Q F G H V F A F G E S
 T A
 AGGTCAITGGCAATAGCCAATATGGTACTTGGGTAAAGAGATCCAAATTCGTGGAGAGATACCGCAATATGTTGACTTACACTTGGAT 900
 R S L A I A N M V L G L K E I Q I R G E I R T N V D Y T V D
 A A T C
 CTCTGAATGCTGCGAGTACCGAGAAATATGATTACACTGGTTGGCTAGACAGCAGAAATAGCTATGCGCGTTAGAGCAGAGAGGCC 990
 L L N A A E Y R E N M I H T G W L D S R I A M R V R A E R P
 CCATGGTACCTTTCAAGTTGTGGTGGAGCTCTATATGAAGCATCAAGCAGGAGCTCGAGTGTGTGTAACCGATTATGTTGGTTATCTCAGT 1080
 P W Y L S V V G G A L Y E A S S R S S S V V T D Y V G Y L S
 C T
 AAAGGTCAATACCAAGCAACATCTCTCTGTCTAATTGACTGTACACTGAATATAGATGGGAGCAATATACGATTGAGACAGTA 1170
 K G Q I P P K H I S L V N L T V T L N I D G S K Y T I E T V
 A CG C
 CGAGGTGGACCCCGTAGCTACAAATTAAGAATTATGAATCAGAGGTTGAGGCAGAGATACATTCTCGGAGATGGCGGACTCTTAATG 1260
 R G G P R S Y K L R I N E S E V E A E I H F L R D G G L L M
 T C G T
 CAGTTGGATGGAAACAGTCATGTAATTTACGCGAGACAGAAGCTGCTGGCACCGCCCTTCTAATCAATGGGAGAACATGCTTATTACAG 1350
 Q L D G N S H V I Y A E T E A A G T R L L I N G R T C L L Q
 T A G
 AAAGAGCAGGATCTTCCAGGTTGTTGGCTGATACCGGTGCAAACTTCTTCGGTTTTTGGTCGCGGATGGTTCTCATGTGGTTGCTGAT 1440
 K E H D P S R L L A D T P C K L L R F L V A D G S H V V A D
 T T
 ACGCCATATGCCGAGGTGGAGGCCATGAAATG
 T P Y A E V E A M K M

FIG. 6

Biotin carboxyl carrier protein primers

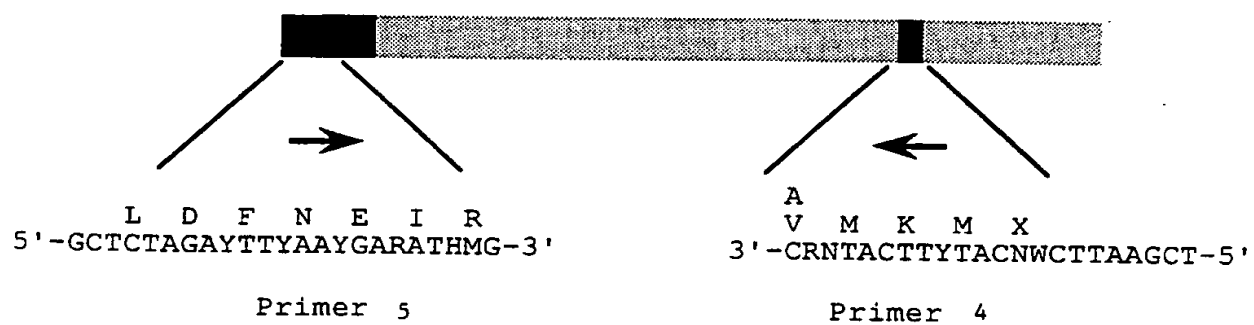


FIG. 7

TCTAGACTTTAACGAGATTCGTCAACTGCTGACAACCTATTGCACAAACAGATATCGCGGAAGTAACGCTCAAAAGTGATGATTTTGAAC 90
L D F N E I R Q L L T T I A Q T D I A E V T L K S D D F E L

AACGGTGCGTAAAGCTGTTGGTGTGAATAATAGTGTGTGCCGTTGTGACAGCACCCCTTGAGTGGTGTGGTAGGTTCCGGGATTGCCATC 180
T V R K A V G V N N S V V P V V T A P L S G V V G S G L P S

GGCTATACCGATTGTAGCCCATGCTGCCCATCTCCATCTCCAGAGCCGGGAACAAGCCGTGCTGCTGATCATGCTGTCACGAGTTCTGG 270
A I P I V A H A A P S P S P E P G T S R A A D H A V T S S G

CTCAGAGCCAGGAGCAAAAATCATTGACCAAAAATTAGCAGAAGTGCTTCCCCAATGGTGGGAACATTTTACCGCGCTCCTGCACCAGG 360
S Q P G A K I I D Q K L A E V A S P M V G T F Y R A P A P G

TGAAGCGGTATTTGTGGAAGTCGGCGATCGCATCCGTCAAGGTCAAACCGTCTGCATCATCGAAGCGGATGAAAAUG
E A V F V E V G D R I R Q G Q T V C I I E A M K M

FIG. 8

INTERNATIONAL SEARCH REPORT

Intern ational Application No
PCT/US 93/09340A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/52 C12N9/00 C12N1/21 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLANT MOLECULAR BIOLOGY. vol. 19, no. 1, May 1992, DORDRECHT, THE NETHERLANDS. pages 169 - 191 SLABAS, A.R., ET AL. 'The biochemistry and molecular biology of plant lipid biosynthesis' see ref. 73 see page 181 ---	13, 16, 17, 27
X	EP, A, 0 469 810 (IOWA STATE UNIVERSITY) 5 February 1992 see column 15, line 45 - column 16, line 40 --- -/--	13, 16, 17, 28-39

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

31 January 1994

Date of mailing of the international search report

22 -02- 1994

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/09340

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOLOGICAL ABSTRACTS, vol. 71 1981, Philadelphia, PA, US; abstract no. 5758, EGIN-BUEHLER, B., ET AL. 'Comparison of acetyl coenzyme A carboxylases (EC 6.4.1.2) from parsley (Petroselinum hortense) cell cultures and wheat germ' see abstract & ARCH. BIOCHEM. BIOPHYS. vol. 203, no. 1, 1980 pages 90 - 100 ---	27
X	DATABASE CAB CAB INTERNATIONAL, WALLINGFORD, OXON, GB an 93:88424 EGLI, M.A., ET AL. 'A 223 kDa subunit of acetyl-CoA carboxylase is encoded by the Accl gene' see abstract & MAIZE GENETICS COOPERATION NEWSLETTER vol. 66, 1992 pages 94 - 95 ---	27
X	DATABASE CAB CAB INTERNATIONAL, WALLINGFORD, OXON, GB an 92:60453 EGLI, M.A., ET AL. 'Purification of maize leaf acetyl-CoA carboxylase' see abstract & MAIZE GENETICS COOPERATION NEWSLETTER vol. 65, 1991 page 95 ---	27
Y	PLANT PHYSIOLOGY. vol. 96, no. 1, May 1991, ROCKVILLE, MD, USA. page 92 EGLI, M., ET AL. 'Purification and characterization of maize acetyl-CoA carboxylase' see abstract 581 ---	13-15
Y	PLANT PHYSIOLOGY. vol. 96, no. 1, May 1991, ROCKVILLE, MD, USA. page 92 EGLI, M., ET AL. 'Purification and characterization of maize acetyl-CoA carboxylase' see abstract 581 ---	13-15
P,X	J. BACTERIOLOGY vol. 175, no. 16, August 1993 pages 5268 - 5272 GORNICKI, P., ET AL. 'Genes for two subunits of acetyl coenzyme A carboxylase of Anabaena sp. Strain PCC 7120: Biotin carboxylase and biotin carboxyl carrier protein' see the whole document ---	1-10, 24-26
1 3	P,X WO,A,93 11243 (ICI) 10 June 1993 see the whole document ---	13-15,27
-/--		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/09340

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 267, no. 2, 15 January 1992, BALTIMORE, MD US pages 855 - 863 LI, S-J., ET AL. 'The gene encoding the biotin carboxylase subunit of Escherichia coli acetyl-CoA carboxylase' see the whole document ---	1-12, 20-26
A	BIOLOGICAL ABSTRACTS, vol. 81 1986, Philadelphia, PA, US; abstract no. 17463, NIKOLAU, B.J., ET AL. 'Use of streptavidin to detect biotin-containing proteins in plants' see abstract & ANAL. BIOCHEM. vol. 149, no. 2, 1985 pages 448 - 453 -----	1-12, 20-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/09340

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0469810	05-02-92	AU-A- 8142191	06-02-92
WO-A-9311243	10-06-93	AU-A- 2953092	28-06-93